THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME IV MAY, 1928 NUMBER 3

CARCINOIDS (ARGENTAFFIN-CELL TUMORS) AND NERVE HYPERPLASIA OF THE APPENDICULAR MUCOSA*

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In 1924, under title of "Neurogenic Appendicitis and Carcinoids," I reported the result of studies commenced in 1913 of more than 400 appendices, some healthy, others with pathological history. I showed that the latter contain neuromata, which perhaps explain the pain of chronic appendicitis. These neuromata arise from the nerves of the periglandular plexus; they form after the migration, into the nerves, of cells containing chromaffin and argentaffin granules which are similar to the granules of the chromo-argentaffin cells of the normal epithelium. I showed further that carcinoids arise from certain intranervous argentaffin cells.

Various considerations lead me to believe that the periglandular plexus from which these neuromata arise is not of sympathetic origin but that it depends genetically on certain entodermic cells and that it represents a *placode*, a *neurentoderm*. This opinion has been rejected as heretical, although the observations on which it is based have been confirmed by many investigators; but no one has been willing to review the question as a whole and with the methods which I recommended.

I return to the subject now because I have controlled my former conclusions by the study of 800 more appendices and because, far from contradicting these conclusions, this study has confirmed them and has enabled me to reply to various objections. I shall report the researches in chronological order; in this way the reader will best understand how and why they led to conclusions which at

* Received for publication February 17, 1928.

first sight seem revolutionary. My warmest thanks are due to my friend, Dr. George F. Laidlaw, who assumed the task of translating this paper for the Journal.

I. Carcinoids

I shall not pause here to review the literature that has been inspired by these curious tumors.* Often multiple, they have been found along the entire length of the alimentary canal from the stomach to the rectum; but their favorite situation seems to be the appendix. Although not strictly identical in every detail, nevertheless, their structure has so many features in common that they may be grouped under the same title, no matter what their situation in the alimentary canal. Thus it is very probable that their point of departure and their causes are the same everywhere. In this paper, I shall consider only the appendicular carcinoids, basing their description on 50 specimens which I have studied personally.

Appendicular carcinoids may form in the body of the appendix where, in growing, they may cause a stenosis of the lumen with retention, followed by acute inflammation. More often, in 46 of the 50 specimens in my collection, they are found at the distal end. If the carcinoid is small, the tip of the appendix is not deformed; if large, it is swollen like a pendulum. This swelling is often the only sign of a possible appendicular tumor but sometimes the presence of tumor is indicated by an opaque, yellowish infiltration of the peritoneal connective tissue and fatty lobules that cover the tip of the appendix.

Section of the appendix shows that the lumen of the tip has disappeared and that it has been replaced by compact tissue, more or less fibrous, hard, yellowish or brownish. To the naked eye the muscular layers seem to be almost intact and always recognizable, even when distended by the tumor and even when the tumor has infiltrated them before involving the peritoneum.

Where the tumor is situated there is no longer an appendicular lumen. The lumen may appear immediately above the tumor; here we may believe that the tumor has caused the obliteration. Sometimes the tumor is separated from the lumen by a variable space where the naked eye perceives nothing but fibrous tissue and

* For the literature, consult the excellent monograph of Wiley D. Forbus, *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 130.

where subsequent microscopic examination reveals no carcinoid cells. This observation is of the first importance for it shows that carcinoids may form in a long stenosed region of the appendix, long deprived of its mucosa and consequently of an epithelial lining.

Histology of Carcinoids: From their structure carcinoids were at first thought to be carcinomata; in fact, they are constituted by columns and masses of epithelia which infiltrate. Their usual benignity and their small size earned for them the name little carcinomata (Lubarsch), then carcinoid (Oberndorfer), under which name they are commonly described. We shall take as a type the carcinoids which form at the tip of the appendix.

When the carcinoids are small, the epithelial columns are situated solely in the axial connective tissue of the appendix; in other specimens, while situated in the axial connective tissue, they invade also the interstices of the circular and longitudinal muscle coats and the nerves of Auerbach's plexus without destroying either the contractile fibers or the nerves. In still other more advanced specimens, they occupy the axis of the appendix, the interstices of the muscle and invade the peritoneal fat. It is obvious that their point of departure is always the axis of the appendix * and that they infiltrate the muscularis and the serosa later. Their development is centrifugal.

Carcinoids consist of epithelial cells grouped in columns of varying breadth and a stroma. We shall study these elements successively in preparations made by the usual methods. The cells are small and of various forms which can be arranged in three groups: round cells, which may be polygonal from reciprocal pressure; palisade cells; and columnar or, better, prismatic cells. Mitoses are rare but not amitotic figures.

Round or polygonal cells are much the most common, forming columns of varying breadth. Those next to the connective tissue are cuboid. The nuclei are central, round and turgid, the network finely reticulated and dotted with minute chromatic granules In well fixed preparations, the protoplasm is clearly bounded by a

* In a paper which appeared in 1914 and which has only recently come to my attention, Ehrlich supposes that carcinoids arise from Auerbach's plexus. On this supposition he bases his statement that these tumors are immature sympathetic neurocytomata. We shall see why this interesting view is inadmissible. For the moment, we note that it rests on insufficient evidence, for Ehrlich saw only advanced carcinoids which had already infiltrated the muscularis and the plexus myentericus.

very delicate membrane. In some cells the protoplasm is homogeneous and frankly acidophil, in others it is abundant and dotted with acidophil granules of extreme delicacy and great regularity. Most of the cells contain tiny vacuoles, sometimes few, sometimes very many and of uniform size like spongiocytes of the adrenal cortex or xanthoma cells (Fig. 29).

Palisade cells (Figs. 28, 30) are less constant. Usually they collaborate with the round cells in forming the columns, ranging themselves along the connective tissue as on a basement membrane. The nuclei present the same texture as those of the round cells; they are turgid, without puckering, and oval in shape. The nucleus is at some distance from the foot of the cell and all this infranuclear region of the cell is filled with acidophil granules, among which may sometimes be distinguished a tiny diplosome surrounded by a nongranular area. This basal and granular region of the cell has no vacuoles but the vacuoles appear about the nucleus and are often very abundant in the supranuclear region.

Sometimes the palisade cells undergo a sort of stratification; some are prismatic, others remain attached to the connective tissue by a long, slender, granular foot while the nuclear and swollen part of the cell insinuates itself deep among the polygonal epithelia. The cell has the form of a tennis racket, only the broad part containing the vacuoles (Fig. 30). Other cells, attached to the connective tissue by a slender foot, are pointed at the other end also, becoming fusiform.

The columns may consist of palisade cells only, curving inward and resembling remarkably the columns of the glomerular layer of the adrenal cortex of certain animals, the horse, for example.

The columnar or prismatic cells are always grouped around a small round cavity or vesicle, forming a rosette (Figs. 28, 31). The cavity may contain a tiny albuminous droplet, more or less colorable, or a homogeneous droplet of colloid aspect. The apical pole of the cell bounds the cavity; this pole is narrow, always bordered by Kittleisten, and consists of a membrane which may be very thin or may be thickened as a striated cuticle. The basal pole rests on the connective tissue or on the polygonal cells. Laterally, the cells are separated by delicate smooth membranes, well outlined. The oval nucleus, similar to those of the palisade cells, occupies the middle of the cell.

The apical pole of each cell is clear, without granules; a tiny diplosome may be detected near the tip of the cell. In some specimens all this supranuclear region is vacuolated. The basal pole is always loaded with granules and is without vacuoles.

These cell forms are connected by all possible intermediary forms and they are often associated in the same column. It is obvious that they do not represent distinct species but rather forms of differentiation of one and the same strain, characterized by nuclei of special structure and by protoplasm which is fundamentally clear and only slightly colorable, in which are often found very tiny acidophil granules and vacuoles, at times numerous but always small and irregular. Moreover, these inclusions which are diffused through the polygonal cells are clearly polarized in the palisade cells. The granules accumulate especially in the basal or connective tissue pole, the vacuoles in the opposite pole. The columnar cells are still more clearly oriented, for the basal pole alone contains granules while the apical pole never has them. Thus the palisade cells and the columnar cells have a granular basal or connective tissue pole and a homogeneous vacuolated apical pole. This orientation is particularly striking in certain specimens where massive columns are penetrated by fine blood capillaries; each of the capillaries is surrounded by a radiating rosette formed by the granular bases of the cells implanted on it.

Beyond the vascular areas just described, the stroma of carcinoids is fibrous or fibro-hyalin, very rich in delicate elastic fibers. There are found also arterioles and venules with muscular walls which scarcely exist in the stroma of carcinomata.

Finally, in that portion of the tumor which develops centrally, inside of the muscular wall of the appendix, smooth muscle fibers are often found among the connective tissue and elastic fibers of the stroma. These may appear in such quantity that they alone form almost all of the stroma, to such a degree that the tumor may be called an adenomyoma or myocarcinoid. Highly important is the fact that this myomatosis does not arise from hyperplasia of the longitudinal or circular layers of the muscle coat; there is always to be found a purely fibrous layer derived from the submucosa which isolates it from the muscularis.

The myomatosis has but three possible sources, hyperplasia of the muscle coat of the arteries or veins, production of muscle fibers

by the connective tissue cells of the stroma, or hyperplasia of the muscularis mucosae. I am inclined to accept this last hypothesis; for the myomatosis is found only in the axis of the appendix, the situation of the fibers of the muscularis mucosae, and not in the longitudinal or circular layers of the muscle coat or in the peritoneal fatty connective tissue when this is invaded by the carcinoid epithelia. In short, the carcinoids seem to have an elective action on the proliferation of the muscularis mucosae.

To sum up their histology, carcinoids resemble ordinary carcinomata of the intestine in the cylindrical form of certain cells; they differ by vacuolation and by the fine granulation of their protoplasm, the granules often accumulating in the vascular pole of the cell.

The Nature of Carcinoids: In 1912, Saltykow, struck by this vascular arrangement of the granules, by their acidophilia and by the endocrine structure of carcinoids, believed them to be tumors arising from aberrant islands of Langerhans. This explanation should never even have been considered for as early as 1907 Oberndorfer had demonstrated that the granules of carcinoids are chromaffin and that their vacuoles are filled with doubly refracting lipoids. Now, the fine granules in the cells of the islands of Langerhans are not chromaffin and these cells never contain doubly refracting fats.

In 1910, Huebschmann advanced the hypothesis that the carcinoids arise from the cells of Paneth or, more probably, from the granular cells discovered in the intestinal epithelium by Nicolas, rediscovered by Kulchitzky, studied later by Schmidt (yellow cells), then by Ciaccio (enterochromaffin cells); but he went no further.

Specific Vacuoles and Granules: On beginning my own studies of carcinoids I found the vacuoles and the granules. In frozen sections the vacuole is seen to be filled by a droplet which stains bright red with scarlet red and with Sudan. The droplets themselves are not doubly refractive but contain crystals which look bright with crossed Nicol prisms. It is probable then that the droplets consist of a mixture of neutral fats and cholesterin esters. Ciaccio's method shows that the walls of the vacuoles contain a very small quantity of lecithin.

From this point of view the fatty inclusions of carcinoids recall those of the cells of the adrenal cortex. On the other hand, the granules are chromaffin and stain black with iron hematoxylin like those of the adrenal medulla. Thus, by the contents of their vacuoles

and by the chromaffin and siderophil reactions of their granules, the carcinoid cells resemble both the cells of the adrenal cortex and those of the medulla and present the features of both in one and the same cell. Now, it is acknowledged that from an embryological, an anatomical, and a functional point of view the adrenal medulla and cortex, the one of sympathetic origin, the other of coelomic origin, are absolutely distinct. Moreover, neither the adrenal nor its tumors ever present prismatic cells grouped in rosettes around a cavity filled with colloid. Therefore, the carcinoids cannot arise from adrenal inclusions.

Next resorting to a new technique which I had already used in the study of melanin, sections of material fixed in the picro-formol of Bouin were immersed in Fontana's ammoniacal silver nitrate. I found that the granules of the carcinoid cells stained dark brown and consequently possessed argentaffin and silver-reducing properties. Sections of adrenal medulla treated in the same way remained colorless, which observation completed the proof that there is no possible identity between carcinoids and the adrenal paraganglion. But this technique applied to sections of the intestine brought out with great clearness the granules of the cells of Kulchitzky.

Thus it became almost certain that the carcinoids arose from a pure proliferation of the enterochromaffin, argentaffin and silver-reducing cells. Since the structure of these tumors resembles that of certain endocrine glands (Saltykow), I called them endocrine tumors and suggested that the chromo-argentaffin cells of the intestine constitute a diffuse endocrine gland.

Since my studies of 1914, a number of researches have been made on carcinoids and on the chromo-argentaffin cells of the intestine. Concerning carcinoids, the opinions may be divided into two groups according to whether or not the writers were familiar with my publications. Thus Abrikossow, following Aschoff, believes them to be nevi of the intestinal mucosa. Krompecher holds them to be basal epitheliomata. Schober groups them among the "progonoblastomata" of Mathias. Engel and Lauche attribute them to embryonic inclusions. Ehrlich makes of them "immature neurocytomata" of the sympathetic.

Those writers who have been willing to adopt my silver technique or any method in which formol fixation is followed by immersion in an ammoniacal silver salt (Delbet and Herrenschmidt, Danisch,

Hasegawa, J. F. Martin, W. D. Forbus, Sprafke), have agreed with me that these tumors arise from the chromo-argentaffin cells of the intestinal epithelium.

As for their endocrine nature and the endocrine function of the cells of Nicolas-Kulchitzky, most writers have refused to follow me. In repeating my experiments on the cells of Kulchitzky, histologists and pathologists have confirmed most of the observations made by my forerunners and by myself; but they have interpreted them in a different manner. Let us study the cells of Kulchitzky as they may be observed in the lining of the alimentary canal.

II. The Cells of Nicolas-Kulchitzky

Yellow cells (*Schmidt*); *enterochromaffin cells* (*Ciaccio*); *argentaffin or silver-reducing cells* (*Masson*); *chromo-argentaffin cells* (*Cordier*): The cell of Nicolas-Kulchitzky is characterized chiefly by its granules and secondarily by a special form and structure; for, under certain circumstances and perhaps in certain functional states, the granules may be absent. Post-mortem changes destroy the granules more or less completely, hence the necessity of using strictly fresh material.

The fixative should always contain formol but no alcohol (Masson, old but unpublished studies; Hamperl, Cordier). Formol itself fixes the granules well but fixes the tissues very badly.* The best fixatives are Bouin's picro-aceto-formol and the bichromate-formol mixture. Bichromate gives to the granules a yellowish brown tint; after picro-formol or formol alone, the granules are invisible because of their special refraction but they stain intensely with ammoniacal silver nitrate, with iron hematoxylin and with acid dyes, such as eosin, acid fuchsin and ponceau de xylidine, as will be described more fully in the section on Technique.

The alimentary canal of all vertebrates contains granular cells similar to the cells of Nicolas-Kulchitzky. In mammals, they are found from the cardia to the anus (Masson, unpublished studies; Hamperl, Cordier), the form varying somewhat with the species and the region. In the gastric glands and in Brunner's glands, these cells insert slender prolongations between the bases of the gland cells; in the human intestine, and consequently in the appendix, their characteristics are as follows (Fig. 1).

* I am perhaps alone in this opinion but the greater my experience the more I am convinced of its truth.

They are scattered singly among the cylindrical cells of the intestinal epithelium, from five to ten in each gland of Lieberkühn, where they are most numerous. They are less frequent in the upper part of the tubule but are found as far as the tip of the villi, where they may be seen desquamated like the other cells of the intestinal mucosa. It is probable that those deep in the tubule are fixed and that only those placed more superficially migrate with their neighbor cells and disappear like them.

In form, the cells of Nicolas-Kulchitzky are almost always conical, with a broad base in contact with the glandular basement membrane, and with a narrow apex bounded by a delicate membrane which is surrounded by the apical membranes of adjacent cells (cells of Paneth, cylindrical cells, caliciform cells). Some of them border the gland lumen, the bulk of the cell being buried among the other epithelia.

The nucleus is round or, more often, oval, turgid and regular in outline except that in some cells the basal pole is hollowed like a cup. The nucleus is never in contact with the basement membrane but rather in the middle of the cell. The nucleus is clear; it contains one or two karyosomes and a network finely dotted with chromatin.

The protoplasm is clear and homogeneous, staining less deeply than the other epithelial cells. There is a Golgi apparatus in the supranuclear region (Cordier) and another in the basal region (Kull). The basal region almost always contains tiny granules which are acidophil, chromaffin, stain with Heidenhain's iron hematoxylin, reduce silver and turn greenish with basic blues. The granules vary in quantity; sometimes they are all beneath the nucleus, sometimes beneath and at each side of the nucleus. Rarely a few are found above the nucleus but never outside of the cell in the intestinal lumen (Cordier). They seem to disappear at certain functional stages of the cell (Cordier).

The granules present a different appearance according to the technique employed. If blocks of tissue fixed in bichromate are treated with silver before embedding (see Technique), the granules are comparatively large, all of the same size and equally black. In paraffin sections treated with silver the granules vary greatly; some of them reduce silver strongly or stain intensely with iron hematoxylin and among these granules some are large and others barely visible; other granules stain neither with silver nor with hematoxy-

lin but with acid dyes. This variable staining may be seen in one and the same cell and also in different cells of the same gland of Lieberkühn, some cells containing more black granules, some more acidophil granules.

In my opinion, these differences in size and staining in tissue treated with silver before or after embedding, depend on the dissolving out by the toluol of some substance which at a certain stage forms part of the granule. Of the nature of this substance, many guesses might be made. Doubtless these variations correspond to different stages of evolution of the granules. From this standpoint it is well to note them but more than this it is impossible to say.

Besides the granules, the basal protoplasm may contain one or two vacuoles, the contents of which stain with Sudan (Danisch).

Thus, in every feature, the cells of Nicolas-Kulchitzky are identical with the cylindrical and argentaffin cells which group themselves as vesicles in the carcinoids. The palisade, fusiform and spongy cells of these tumors are not found in the normal epithelium; doubtless they represent a morphological deviation which depends on their peculiar situation in the interstices of the connective tissue.

Origin of the Normal Argentaffin Cells: My embryological studies, confirmed by those of Parat, have shown me that the argentaffin cells appear in the intestinal epithelium about the fourth month of fetal life in man; they seem to spring directly from the cells of the entoderm. Kull, however, studying them in the chick embryo by a mitochondrial method, not by ammoniacal silver, derives them from mesenchyme cells that have invaded the epithelium. This opinion cannot be maintained; the very figures published by Kull forbid it. There is not the slightest possible resemblance between the mitochondria of the connective tissue cells of the mucosa and the chromaffin granules of the chromo-argentaffin cells, although these latter also stain with Altmann's acid fuchsin. Their dimensions are very different.

Influenced by my own work (see Argentaffin-cell Neuroma, page 192), Danisch seeks to show that the chromo-argentaffin cells arise from the solar plexus and migrate to the intestinal epithelium in the fourth month of fetal life. His figures are not very convincing and, according to his own statement, represent macerated tissue. Moreover, Danisch uses Agdhur's silver technique which indeed colors the argentaffin cells but not specifically and colors also certain

chromaffin cells of the sympathetic paraganglia. His conclusions should be rejected.

There are two pathological observations which tend to show that the chromo-argentaffin cells are really of entodermic origin:

1. In chronic gastritis, they are found in abundance in those glands of intestinal type which form in regeneration of the mucosa.

2. They are sometimes found (Hamperl, Martin and Masson) in cancers of the intestinal tract and in their metastases, mixed with ordinary cylindrical cells, and may be traced easily to their origin in the common type of cylindrical cell.

Function of the Argentaffin Cells: The granules of the cells of Nicolas-Kulchitzky have inspired many physiological hypotheses. Ciaccio holds that, because of their chromaffinity, the cells produce adrenalin which is poured into the intestinal canal. However, the specific reaction to ammoniacal silver indicates that the substance secreted differs from adrenalin. The chromaffinity and the silver reduction are proofs of reducing power; more than this cannot be said at present.

The situation of the granules at the base of the cell indicates an endotropic polarization of the cell; for this reason I have advanced the idea that they have an endocrine function. In their normal state this function is doubtless not exclusive. Kull states that the cells have two Golgi apparatuses, one apical, the other basal. According to Cowdry, the situation of the Golgi apparatus is connected with the pole of discharge of the cell. Moreover, in the carcinoids, the presence of cavities of secretion bordered by cylindrical cells and filled with an albuminous or colloid liquid, demonstrates that these cells secrete something from their apical pole; in a word, they are exocrine.

However, this does not exclude a concomitant endocrine function. Liver cells normally exhibit two polarities, external and internal. The pancreatic cell is capable of exhibiting them successively and, to return to the carcinoids, it is obvious that if the palisade and the polygonal cells of the larger masses secrete anything, either they retain it in their interior or eliminate it into the vessels. If then the cylindrical argentaffin cells of the normal intestine and of the carcinoids secrete in both directions, the cells of the same lineage which do not border the lumen of a tubule have certainly lost their exocrine function and can be only endocrine. This endocrine function is,

perhaps, not without some connection with the proliferation of the muscle fibers which is so often observed in the stroma of carcinoids.

Cordier has expressed doubt of the endocrine function of the cells of Kulchitzky; in asserting their exocrine function, he relies on the disappearance of the granules after injection of pilocarpin. I do not deny this disappearance but it proves nothing against my thesis; for Cordier has been unable to show in which direction, intestinal cavity or interstitial tissue of the mucosa, the product which results from the destruction of the argentaffin granules is excreted.

In sum, our knowledge of the normal chromo-argentaffin cells shows their identity with the cells which constitute carcinoids; but their physiological rôle remains obscure and their endocrine function in particular remains to be proved.

III. Argentaffin-cell neuroma

(*A*) argentaffin-cell neuroma in obliterated appendices: If carcinoids are really endocrine tumors composed exclusively of enterochromaffin and silver-reducing cells, there is reason to believe that the normal enterochromaffin cells, when situated in the intestinal epithelium, possess similar properties and functions, and that these are endocrine. How shall we verify this?

There comes to mind Laguesse's famous experiment in which he demonstrated the endocrine function of the islands of Langerhans by ligating the external pancreatic ducts. The exocrine acini atrophied rapidly; the islands persisted. At one stroke, he demonstrated their purely endocrine function and their rôle in the metabolism of glucose by the elaboration of a substance which has since received the name insulin. Unfortunately, an experiment of this kind cannot be carried out on the intestine, especially the appendix, ligature of which would be followed immediately by subacute inflammatory symptoms. At this point, I thought of utilizing the experiments performed by nature and examined appendices in which the lumen had been obliterated by cicatricial stenoses, or such as were so considered by most writers, in the hope of finding argentaffin cells in that axial connective tissue which replaces the former lumen and the mucosa.

If the accidents of cicatrization had incarcerated fragments of the glands of Lieberkühn and isolated them from the intestinal cavity,

the argentaffin cells, if really endocrine, might survive the others and persist in the connective tissue just as the islands of Langerhans persist after disappearance of the acini.

My first studies in this direction did not confirm this anticipation but they enabled me to draw three unexpected conclusions:

1. In the axial connective tissue of obliterated appendices there is always to be found a discontinuous, more or less prominent, muscular sheath formed by the persistence of the smooth muscle fibers of the muscularis mucosae.

2. Inside of this sheath there are always nerves, non-medullated, always large and often clustered together to form neuromata, included in connective tissue which is sometimes fibrous, sometimes edematous, sometimes hollowed out by an axial lacuna.

At first, I believed that these neuromata, so frequent in obliterated appendices, were amputation neuromata similar to those of the cerebrospinal nerves and that they were caused by division of sympathetic filaments of the mucosa by an ulcerative process. About this time, Maresch made similar observations and within a few days of each other we published almost identical papers on appendicular neuromata considered as amputation neuromata.

3. At the time when my work appeared, this explanation did not satisfy me altogether. In fact, in pursuing my studies, I had ascertained that the nerves which persisted in the appendicular axis and especially in the neuromata always contained cells, the protoplasm of which was dotted with argentaffin granules. Thus the long-sought cells were found again but this time inside of the nerves and never outside of them in the interstitial connective tissue.

This observation, the constancy of which I was able to verify, seemed to me to be of fundamental importance. It no longer permitted the interpretation of the appendicular neuromata as common amputation neuromata, for these do not contain argentaffin cells. From another point of view, it might be asked if carcinoids and argentaffin-cell neuromata were not related and even if they had not a common anatomical basis.

The Neuromata: The neuromata are composed of compound and plexiform non-medullated fibers identical with those of the intestinal mucosa, but voluminous, rolled up in masses and in close apposition. The trichrome stain colors their collagen sheath blue, the neuroglia bright red. The neuroglia is formed of ramifying and

anastomosing tubes, the contents of which, consisting doubtless of neurites, stain pale pink (Figs. 21, 22, 23, 24, 25).

The neuromata vary greatly in size. Some are microscopic, some large enough to be visible to the naked eye. They may attain a diameter of from two to three millimeters without piercing the sheath formed by the vestiges of the muscularis mucosae. Only twice have I seen neuromata which had passed this usual boundary and broken through not only the muscularis mucosae but the external muscle coat as well.

The neuromata are usually numerous; the smaller the tumors, the more easily counted. I have counted from 50 to 60 in one centimeter of appendix! When large, they tend to fuse in masses or the nerve fibers interlace in an inextricable tangle. It is important to note that they are always connected with one another by plexiform fibers, some transverse and oblique (Fig. 23), linking neuromata which have formed at the same level, others longitudinal (Figs. 23, 25), connecting tumors at different levels. In some well-oriented, longitudinal sections the appearance of these neuromata linked together recalls the nerve chain of an arthropod or worm.

Besides these interconnections the neuromata anastomose laterally and externally with Meissner's plexus of the submucosa which, however, exhibits no increase of ganglion cells or fibers.

The appearance of the neuromata varies in different specimens. Some are formed of slender fibers, rich in Remak's nuclei; these are evidently in active growth. Others consist only of large fibers with scanty nuclei; these seem to have completed their growth (Fig. 22). Others again are invaded by lymphocytes; the fibers are widely separated, shrunken and clearly in full retrogression (Fig. 21).

It is probable that the life of these neuromata is ephemeral and that they disappear one after another more or less completely; but this solitary retrogression and lymphoid infiltration would occur only if each of them had a certain autonomy in spite of its connections with its companions and with Meissner's plexus. Almost every obliterated appendix examined contained at least vestiges in the form of longitudinal and axial nerve fibers.

Are these structures really nerves and neuromata? If the answer is sought in Cajal's or Bielschowsky's technique of silver impregnation and reduction, these are not neuromata; for silver never colors neurites in them. All my attempts in this direction have failed,

as well as those of Schweizer and other workers. Schweizer concluded that these supposed neuromata are nothing but neurinomata (Schwannomata); others who, disregarding my warning, have used only the silver-reduction methods in studying obliterated appendices, have seen no nerves whatever and have simply denied my statements (Lauche).

Silver impregnation is the most capricious of all histological methods. On occasion, it colors neurites, neuroglia, elastic fibers, reticulum, collagen, we know not how or why. The many modifications of silver technique suffice to show its unreliability. As for coloring neurites, outside of the central nervous system the results are so inconstant and imperfect that I refuse to accept them as characteristic. When silver reveals black fibers in the nerves, I believe indeed that they are neurites; if silver shows nothing in a tissue which other methods, the trichrome stain especially, stamp as nervous, I reject the negative evidence. Moreover, as we shall see later, if the mucous membrane of the normal appendix is treated by Cajal's or by Bielschowsky's method, very few neurites take the stain, although the trichrome stain reveals an abundance of nonmedullated fibers. It must be conceded then, not that there are no nerves in the intestinal mucosa, but that the greater number of nerves in the intestinal mucosa escape silver impregnation; now, by their topography, as we shall see, the neuromata of the obliterated appendix belong to this plexus of the intestinal mucosa.

There is another argument against the neurinoma hypothesis. All neurinomata with which we are familiar arise from localized proliferation of the Schwann cells; they contain no argentaffin cells; they are independent of one another; they do not regress. The nerve tumors under consideration communicate by longitudinal fibers, uniting one with another, and they regress individually as if, in spite of their continuity, they enjoy a certain autonomy. Hence the hypothesis that they are true neuromata, just as the nerves of the mucosa are true nerves notwithstanding the absence of argentophil neurites; and that their trophic center is neither Meissner's nor Auerbach's plexus, neither of which participates in their growth or in their degeneration.

Argentaffin cells: Argentaffin cells are always present in the growing or fully developed axial neuromata of the obliterated appendix, either singly or in groups of from two to twenty cells of the most

varied forms. Some are polygonal from reciprocal pressure, or more or less rounded.

They are enclosed in a neuroglial syncytium but their external limits cannot be determined exactly. They seem to form a part of the syncytium. Their nuclei are similar to those of carcinoid cells. Their cytoplasm is loaded with silver-reducing chromaffin, siderophil and acidophil granules, and often hollowed out by vacuoles containing lipoids. The droplets escape from the cell bodies like a product of secretion and disappear in the neuroglia. To this peculiar process of intranervous internal secretion I have given the name *neurocrinia*. Cells which belong to this type I call *neurocrine cells* (Fig. 11).

Other cells have no distinct cytoplasmic body; their nuclei are enclosed directly in the neuroglial syncytium and are surrounded by a few silver-reducing granules. These argentaffin cells are no longer individualized, they form an integral part of the Schwannian syncytium (Figs. 9, 10).

Other cells have the clear contour of ganglion cells, the nucleus at times vesicular, poorly chromophil and provided with rounded nucleoli. Only their silver-reducing granules identify them as argentaffin cells. Basic blues may show angular figures resembling Nissl bodies. I call them *cells of ganglion type* (Fig. 10).

Still other cylindrical or cuboid cells are arranged in a rosette around a cavity filled with a substance of colloid appearance, the whole being enclosed in the neuroglial syncytium. Only their basal region is filled with the silver-reducing granules. These cells correspond to the cells of Kulchitzky; they are *cells of the intestinal type* (Figs. 9, 10).

In short, in these neuromata we find cylindrical argentaffin cells and lipo-secreting cells as in carcinoids; but besides these we see two other types, which belong to the same strain however, argentaffin cells incorporated in the Schwannian neuroglia and argentaffin cells of ganglionic aspect. All these cells are in intimate contact with the neuroglial cytoplasm and completely separated from the interstitial connective tissue.

There is still more. If we study serial sections of neuromata in regression infiltrated with lymphocytes we never find argentaffin cells of any kind; but they are always to be found in the other neuromata. This observation together with the obvious indifference of

the ganglion cells and nerves of Meissner's and Auerbach's plexuses to the growth and degeneration of the axial neuromata of the appendix, leads me to believe that these neuromata depend exclusively on the multiform argentaffin cells contained in their fibers.

Without my former studies on carcinoids and the cells of Kulchitzky, I should have believed these neuromata to be ganglioneuromata of the sympathetic. I should have accepted the polygonal cells as paraganglionic and the cells of the ganglion type as sympathetic. As for the rosettes, I should have held them to be neuro-epithelial. Unfortunately, this latter interpretation could not have been advanced without embarassment; for the embryonic sympathetic contains no hollow rosettes with colloid contents, bordered by cells with a striated cuticle but rather groups of sympathogonia without central cavities. The rosettes, the silver-reducing granules and the colloido-secretory cells give to the neuromatous complex an especial character which is not seen in cerebrospinal ganglioneuromata or in sympathetic ganglioneuromata. On the contrary, they are too like the normal cells of Kulchitzky and the carcinoid cells to neglect to inquire into their possible intestinal origin.

(*B*) NEUROMATOSIS OF THE PERMEABLE APPENDIX: *Origin of the neuromata of obliterating appendicitis:* It was at this point that I took up the study of appendices still provided with a mucosa. It was obvious that if argentaffin cells occur in the neuromata, they had migrated into the nerves while the epithelium of which they normally form a part still existed. I began by examining healthy appendices or those supposed to be healthy, without pathological history, excised during laparotomies performed for various reasons, and then realized the peculiar richness of the periglandular nerve plexus of the appendix, as shown by the trichrome stain. This richness is maximum between the ages of 18 and 35 years and is far greater than in any other part of the intestine. The nerves of this plexus ramify in direct contact with the gland tubules deep in the reticulated tissue of the mucosa.

I made this observation also, that ammoniacal silver nitrate nearly always reveals an occasional argentaffin cell in these nerves. Their detection may require prolonged search for there may be only one cell in thirty or forty serial sections. As already stated, I applied the methods of Cajal and of Bielschowsky to these nerves without success.

Turning to appendices with pathological history but extirpated in the absence of any acute inflammatory crisis, I found almost always an abnormal number of silver-reducing cells in the nerves of the periglandular plexus (Fig. 13), and exceptionally a few in the inner layer of Meissner's plexus. These cells may be very few and found only after diligent search, there being only one or two in four or five sections of 5 microns; in other specimens, they are innumerable, either scattered or massed together, always inside of the nerve filaments and never in the interstices of the lymphoid tissue (Fig. 2).

In the periglandular nerves, these cells have the same characteristics as those already described in the neuromata; they may be neurocrine, Schwannian, ganglionic or cylindrical. These latter are not constant, the neurocrine cells always predominating.

Finally, the nerves inhabited by the cells are broadened, hypertrophied and increased in number. In some specimens they form actual neuromata, diffuse or localized, which push the muscularis mucosae before them (Figs. 16, 17). Their fibers are closely approximated, narrowing the meshes of the reticulum. Such a neuromatous mucosa has a characteristic clearness owing to a diminution in the number of lymphoid cells.

The neuromata are often multiple. They are situated solely beneath the gland tubules, never in the lymphoid follicles. They are connected with Meissner's plexus by non-hypertrophied fibers which traverse the muscularis mucosae. In these neuromata a certain number of neurites may be impregnated with silver, especially about their connections with Meissner's plexus, but many of them take the silver no more readily than do the neurites of the normal gland plexus or of the neuromata of the obliterated appendix.

A step further, in studying *incompletely obliterated appendices,* I found that their neuromata were continuous with the plexus of the mucosa and that they represent an extension of the argentaffin-cell neuromata which had developed in the still permeable portion of the mucosa.

Thus the neuromata of obliterating appendicitis arise from the hyperplastic plexus of the mucosa, this plexus being inhabited by argentaffin cells. If the plexus contains the argentaffin cells, it will survive and undergo hyperplasia even when the epithelial lining of the appendix has disappeared.

Origin of the Intranervous Argentaffin Cells: It remained to dis-

cover the source of the intranervous argentaffin cells. The first thing that struck me was that no matter how great their abundance, I had never seen one in mitotic or amitotic division. Whence could they come if not from the epithelial lining? Minute examination showed me that it was indeed thus and that all the argentaffin cells enclosed in the nerves result from intranervous budding from the glands of Lieberkühn. The budding epithelia then separate from their gland matrix, migrate into the nerves of the mucosa and differentiate into the various types already described.

Budding: To demonstrate argentaffin cells in the nerves of the plexus of the mucosa is an easy task if the observer is willing to use my technique, ammoniacal silver nitrate and the trichrome stain. To trace their migration from the Lieberkühnian lining into the nerves is much more difficult and rarely possible. This will be readily understood on reviewing the conditions that must happen to coincide so that a process of such short duration and of such capricious orientation may occur at the precise moment of tissue fixation, in sufficient abundance to exhibit the various stages and in a direction that coincides with the plane of the sections. I should add that the process is momentary, that it occurs under circumstances beyond our control, usually during appendicular colic with slight inflammation, and in subjects from 18 to 25 years of age. Of the 1200 appendices examined, I found budding in only fifteen. To give an idea of the difficulty of this work, I may say that in those specimens in which the buds were most numerous, I counted on an average only one in seventeen serial sections of 5 microns thickness. That is to say, finding the buds is a matter of perseverance.

I trust that I may be pardoned for giving these details. The object is not to discourage those who may wish to control my observations but to warn them against the skepticism which might follow researches insufficiently pursued.

Figures 3, 4, 5, 6, 7, 8, 15, make unnecessary a long description of this process. Budding begins with an increase in the number of nuclei in a limited area of the lining of a gland of Lieberkühn. The multiplication of nuclei is always amitotic. It is strictly localized, either at the tip of the gland tubule or more often a little to one side. It is never seen in the annular zone where mitotic figures are found or above this point.

The nuclei invariably belong to the *indifferent cells* and in the

beginning preserve their irregular contours and customary flabby aspect. Their multiplication obliges them to arrange themselves in several layers, some in the middle zone of the cell, some close to the basement membrane. Granules are never seen in the cytoplasm of these cells the limits of which are so indistinct as to give to them as a whole a syncytial aspect.

The basal ends of two or three adjoining cells now elongate, uniting to form a small projection which first pushes before it and then perforates the basement membrane and at last finds itself directly in contact with a nerve filament of the periglandular plexus (Figs. 3, 4).

Several nuclei now migrate into this pseudopod which elongates and buries itself in the nerve. The epithelial bud thus formed may remain small, consisting of few cells, or on the contrary it may grow large and multicellular. In the latter instance it assumes a spherical form and remains for a time attached to the gland by a slender pedicle. It is then and then only that the appearance of the cell changes. The nucleus swells, the chromatin becomes regularly and finely reticulated. Silver-reducing granules appear abruptly in the cytoplasm. These changes are observed first in the cells which have pushed farthest into the nerve, the first to emigrate, then in the others (Figs. 5, 6, 7, 8).

Soon the pedicle of the bud shrinks, parts, and the cells find themselves cut off from the original gland tubule. In some lucky sections in which the tissue happened to be fixed shortly after the division of the pedicle and where the invaded nerve has been cut longitudinally, a group of argentaffin cells may be seen isolated in the nerve a few hundredths of a millimeter from the lining of the gland tubule. This nerve is in direct contact with the Lieberkühnian cells which remain in the tubule and these cells insinuate slender prolongations which blend with the neuroglia (Fig. 15). Intestinal cells and neuroglial syncytium are continuous. Such pictures have a further value; they show that the various views of budding represent the emigration of epithelial cells into the nerves and not the immigration of formerly intranervous cells into the gland tubule.

Migration: After their separation from the intestinal epithelium, the cells which have become argentaffin may remain in the vicinity of the parent gland. More often they continue their migration inside of the filaments of the subglandular plexus and by this path

reach a deeper level of the mucosa. Rarely do they reach the muscularis mucosae or the superficial branches of the submucous plexus, never beyond this point.

Migration is preceded by dislocation of the cells of the bud which, when liberated, seem to insinuate themselves between the nerve fibers. Their advance is checked here and there at a crotch of the plexus by the interlacing nerve fibers. The ganglion cells (Fig. 14), present a particularly insurmountable obstacle. When budding is very active and frequent, the nerves beneath the budding gland may be seen to be invaded by argentaffin cells which, having been separated for a time, now range themselves in rows or pile up in masses as if all of them had been arrested by the same obstacle. It is in these masses resulting from blocking of the cells at certain points, and not by multiplication that the cells adapt themselves to one another and form vesicles or rosettes with colloid contents (Figs. 8, 9, 10, 12).

Differentiation of the argentaffin cells: In recent cell groups we may observe differentiation into one or the other type, intestinal, neurocrine, ganglionic or neuroglial (Figs. 9, 10). This phase of differentiation of the intranervous argentaffin cells is short, apparently as short as the phase of glandular budding which preceded it. It is seen clearly only in rare instances where budding has been met in great activity. Budding terminated, each emigrant cell soon assumes its type. The submucous plexus is crowded with polymorphic argentaffin cells, all isolated from the epithelium and capable of persisting for a long time, doubtless for years. It is in this stable form that we most frequently find the intranervous argentaffin cells in chronic, non-obliterating appendicitis and in the axial neuromata of the obliterated appendix.

Beginnings of nerve hyperplasia: In budding appendices, the periglandular plexus presents great inequalities in the caliber of the nerve fibers, the most voluminous being precisely those which have been invaded by the argentaffin cells. The nerves are enlarged not only at the points occupied by the argentaffin cells (Figs. 7, 8, 9, 10, 11, 12) and especially by the cell masses, but also beyond the cell masses where they are constituted by nerve fibers only. The enlargement of the nerve is due to an increase in the number of its constituent fibers, a multiplication due doubtless to the presence of argentaffin cells. The hyperplasia may remain limited to a few nerves or it may in-

volve a whole region of the periglandular plexus; it may not only enlarge the fibers already existing but also produce new ones which interlace, thicken and end by forming the neuromata already described.

The growth of the nerve fibers is not attended by proliferation of the argentaffin cells which become more widely separated as the neuromata are more voluminous. There is, however, an abundant amitotic multiplication of the neuroglial nuclei, Remak's nuclei, which increase in number in proportion to the increase of the neuromatous mass. The growth remains strictly limited to the intramucous plexus; the submucous and the myenteric plexuses do not participate in any way, save for rare exceptions which I shall describe in another paper.

The emigration period: In my own experience, as already related, gland budding, precursor of nerve hyperplasia, does not take place at all times. All of the subjects on whom I made these observations were between 18 and 25 years of age, the period during which, normally, the periglandular plexus has its maximum growth. Furthermore, all of these appendices presented slight inflammatory lesions involving the mucosa or had given rise to symptoms of slight acute appendicitis several days before intervention. In order to produce abundant budding it appears that two conditions must coincide, a non-mutilating irritation of the mucosa and the active period of normal neurogenesis.

On the other hand, all appendices, even normal ones, may present an occasional argentaffin cell in their nerves and these cells also are of intestinal origin. Therefore, we may suppose that the large number of buddings observed between the years of 18 and 25 both during and following slight inflammatory crises, and represented in the sequel by the presence of argentaffin cells in great numbers in the nerves, represents an exaggeration, under the influence of an irritation occurring at a favorable moment, of a normal process of neurogenesis too discrete to be detected in the healthy appendix. Thus the excessive neurogenesis which prepares the way for neuromatosis seems to be determined by an ordinary and non-mutilating acute appendicitis.

IV. Origin of Carcinoids

During the study of so many appendices, chance has enabled me to observe many carcinoids. As stated in the beginning of this paper, I have examined 50 of these tumors. Many of them were given me by pathologist friends; all of these were large. In my own material I have found 15, some of which were of particular interest because of their small size. Five were invisible to the naked eye; all had developed in neuromata, either terminal neuromata such as are found in obliterated appendices, or lateral neuromata, contiguous to a mucosa still retaining its epithelium.

In all of these specimens I was able to convince myself (Fig. 27) that the carcinoid cell columns had resulted from proliferation of intranervous argentaffin cells of the neurocrine type. These cells, piled up in the nerve fibers, finally rupture their sheaths and infiltrate the intestitial tissue of the neuroma, then that of the submucosa. These neurocrine cells proliferating in the connective tissue assume the characteristic appearance of carcinoid cells. They still secrete fatty droplets but these accumulate in their cytoplasm making it spongy. This results, perhaps, from the impossibility of eliminating their secretion into the nerves, for they are now buried in connective tissue. Proliferating in such an abnormal medium as connective tissue, the neurocrine cells group themselves like ordinary endocrine cells.

Summary and Conclusions

1. The axial region of completely obliterated appendices often (86 per cent of the specimens) contains nerves and neuromata enclosed by a discontinuous sheath formed by vestiges of the muscularis mucosae. These neuromata always contain argentaffin cells of divers forms. If the argentaffin cells disappear, the neuromata regress and are absorbed individually, notwithstanding their connections with Meissner's plexus of the submucosa.

2. Study of partially obliterated appendices shows that these neuromata arise from the periglandular plexus and that they often continue the neuromatous evolution which had commenced when the mucosa still contained gland tubules.

3. This periglandular and subglandular neuromatosis always occurs in nerves inhabited by argentaffin cells.

4. The intranervous argentaffin cells spring from the epithelium that lines the bottom of the glands of Lieberkühn.

Reversing the order in which these observations were made suffices to reconstitute the probable and logical chronological order of the phenomena. Certain intestinal cells bud out and migrate into the nerves. Here they become argentaffin. They differentiate into various forms, cylindrical cells grouped in rosettes or vesicles, cells of ganglion type, of Schwannian type, neurocrine cells. The nerves containing them grow and become neuromata, or disappear if the cells themselves disappear.

Carcinoids result from the autonomous proliferation of the isolated neurocrine cells present in the neuromata. In short, by its neuromata and by the argentaffin cells which determine their growth and which are linked with their persistence, the periglandular plexus of the appendix exhibits a remarkable autonomy.

If the cells possessed no specific granules and if we were ignorant of their intestinal origin we would be led to believe that some of them were derived from the sympathetic system, ganglionic or Schwannian, others chromaffin and paraganglionic, and the carcinoids, issue of the latter, would be sympathetic paragangliomata as stated by Danisch. However, their silver-reducing granules, their lipoids and their entodermal origin upset this hypothesis and suggest two others:

Either the nerve plexus is of sympathetic origin and proliferates under the irritating or secretory influence of the cells which have emigrated from the intestinal epithelium (but how then shall we explain the diverse morphology of these cells and especially their invariable migration into the nerves,* never into the lymphoid interstices?); or the periglandular plexus consists of a mixture of fibers, some sympathetic, others belonging to another nervous system, autonomous and autochthonous, of entodermic origin. The fibers of this latter system, like the neuromata which spring from

* Van Campenhout has recently shown that phenomena similar to those that I have seen in the appendix take place in the fetal pancreas of various mammals; they consist in emigration of cells from the primitive endocrine islands into the pancreatic nerves. The emigrated cells constitute the "sympathetico-insular complexes" to which the author freely attributes an endocrine function. Without doubt, he is right; but we might inquire if there is not something more and if certain insular cells do not take part directly in the genesis of part of the pancreatic nervous system.

them, are refractory to silver impregnation and thus their precise origin escapes us.

In the normal state might not this origin be in certain cells which are mingled with the glandular and absorbent cells just as the olfactory fibers spring from certain cylindrical and nevertheless ganglionic cells of the pituitary epithelium? From this point of view we could understand their proliferation and their elective migration into the nerves which are already in continuity with them, their forms of differentiation following exclusion from the epithelium, and the growth of the nerves.

Is it forbidden to consider the possibility of a neurentoderm, an entodermic placode, of which the cells of Kulchitzky are the sole manifestation in normal conditions? In this hypothesis, the neuromata of neurogenic appendicitis on the one hand and the carcinoids (paragangliomata of the neurentoderm?) on the other hand would illustrate the complexity of this nervous system which permeates the entire intestine (the ubiquity of carcinoids proves this) but which is not demonstrable with our present technique.

My hypothesis of the autonomy of a part of the periglandular plexus receives indirect confirmation from the demonstration of a neuromatosis which is undeniably linked with the sympathetic system. These neuromatoses are very different from those described in these pages. I shall reserve their description for a future paper.

Technique

Fixation:

Bouin's Fluid

Formol	10.
Water	30.
Glacial acetic acid (or 10 per cent trichloracetic acid)	2.
Picric acid	to saturation

Optimum fixation time: 3 days.

Do not wash in water. Dehydrate immediately in alcohol and embed in paraffin.

Regaud's Fluid

Formol	20.
3 per cent aqueous solution of potassium bichromate	80.

Optimum fixation time: 24 hours.

Wash in running water. Embed in paraffin.

Sections: Sections of 5 microns should be numerous and in series. The lesions to be studied are always localized; we must multiply the chances of finding them and be able to study them in three dimensions. The buds and the neuromata cannot be understood without many serial sections.

Affixing the sections: Dissolve 0.05 gm. gelatin (in practice, a bit of ordinary sheet gelatin one-fourth inch square) in 20 cc. distilled water, warming the water over the flame. Filter a few drops of this solution on the slide and float the section on it. Place the preparation on the warm plate at 40° C. As soon as the section spreads, remove the slide, hold the section in place with a brush or needle and stand the slide upright to drain. Blot with absorbent paper and dry in the oven at 40° C in formalin vapor secured by leaving in the oven an open bottle of formalin.

This is the only method which gives perfect adhesion of the sections no matter what the fixative, the duration of the stain, or the temperature employed.

Staining:

The Trichrome Stain

First stage: Staining the nuclei with iron hematoxylin.

The sections, freed from paraffin by toluol, alcohol and water, are immersed in 5 per cent iron alum previously heated to 45 or 50° C for 5 minutes. Wash in water.

Stain for 5 minutes at 45 to 50° C in Regaud's hematoxylin.

Hematoxylin	1.
Alcohol 95 per cent	10.
Glycerin	10.
Distilled water	80.

Rinse with 95 per cent alcohol.

Differentiate in picric alcohol, which is more selective than iron alum.

Alcohol 95 per cent saturated with picric acid	2 parts
Alcohol 95 per cent	1 part

Wash in running water.

Second stage: Staining the cytoplasm and the collagen.

Prepare the following solutions:

(*A*)	Acid fuchsin	0.3
	Ponceau de xylidine*	0.7
	Distilled water	100.
	Glacial acetic acid	1.
(*B*)	Phosphomolybdic acid	1.
	Distilled water	100.
(*C*)	Glacial acetic acid	2.
	Distilled water	100.
	Aniline blue	to saturation

Stain in *A* for 5 minutes.

Rinse with distilled water.

Differentiate in *B* for 5 minutes.

Without rinsing, pour 10 drops of *C* on the section, tilt the slide a few times for thorough mixing and let stand for 5 minutes.

Rinse in distilled water.

Back to *B* for 5 minutes.

In 1 per cent acetic acid water for 5 minutes.

Dehydrate, clear and mount in salicylic balsam.

Results: Nuclei black; argentaffin granules black or red; cytoplasm and neuroglia vermilion red; collagen intense blue.

SILVERING THE REDUCING GRANULES

(*A*) *Silvering the sections:*

Fixation: Despite the advice of many writers, tissue that is to be treated with silver should not be fixed in potassium bichromate, which in my opinion gives poor results. Formol preserves the granules very well but the delicate cytoplasmic structures very badly. The picro-acetic formol of Bouin is the fixative of choice.

Prepare the ammoniacal silver nitrate solution as follows:

To 100 cc. of a 20 per cent aqueous solution of silver nitrate, add aqua ammonia drop by drop, shaking well, until the precipitate of silver oxide is just dissolved; then add a few drops of the 20 per cent nitrate until there is a persistent opalescence. The fluid should have no odor of ammonia. Add distilled water to 200 cc., the solution now containing 10 per cent of silver nitrate. Keep in a strictly clean glass bottle and filter just before use.

* Of the brand *Microcolor*, 35 rue Escudier, Boulogne-sur-Seine (Seine), France. For this purpose, other brands of ponceau are very inferior.

The sections, affixed to slides with gelatin, are immersed in toluol, alcohol and then in distilled water, then in the ammoniacal silver nitrate at room temperature in the dark.

In 4 or 5 hours, the argentaffin granules turn yellow, then brown. In 24 hours or in 36 hours at the longest, the silver is completely reduced. Within this time limit, the silver is reduced also on certain coarse granules sometimes contained in the macrophages of the reticulum, granules of lipofuscin or of purin products, all very different from the fine granules of the argentaffin cells. Too long immersion colors the nuclei and the connective tissue.

After immersion in the ammoniacal silver for 24 hours or 36 hours at the longest, the sections are washed in water and toned in the gold bath as follows:

(*A*) Ammonium sulphocyanide	6.
Distilled water	100.
(*B*) Sodium hyposulphite	6.
Distilled water	100.
(*C*) Gold chloride	2.
Distilled water	100.

Mix 1 cc. *A* with 1 cc. *B*; add *C* until there is coarse precipitation and pour over the sections. Toning is instantaneous.

Rinse with 6 per cent hypo. Wash in running water.

Result: The argentaffin granules are opaque black.

It is well to stain the background, either with Cajal's picro-indigo-carmine or, better, by the second stage of the trichrome as described above, ponceau-acid fuchsin-phosphomolybdic acid-aniline blue. I recommend the latter especially; for it brings out all the tissue elements perfectly.

(*B*) *Silvering in the block:*

Fix in Bouin for 3 days.

Cut slices 2 to 3 mm. thick; wash them in running water for 24 hours.

Immerse for 24 hours in a solution of 2 drops aqua ammonia in 100 cc. distilled water.

Immerse for 24 hours in the ammoniacal silver nitrate diluted with 3 volumes of distilled water.

Rinse in distilled water.

Tone in Cajal's mixture for 24 hours:

Ammonium sulphocyanide	3.
Sodium hyposulphite	3.
Distilled water	100.
Gold chloride (1% sol.)	1.

Wash in water for several hours, embed in paraffin or celloidin.

All the argentaffin granules are opaque black (see the text); the nuclei are brownish. Counterstaining is unnecessary. It is scarcely necessary to add that the nerves are never impregnated by this method.

SUPPLEMENTARY METHOD

Impregnation of the neurites in appendices fixed in Bouin:

Fix as usual in Bouin for 3 days.

Preserve the tissue in 5 per cent neutral formol for 2 months.

At the end of this time, cut slices from 1 to 2 mm. thick, wash them in pure distilled water for 12 hours and then for another 12 hours in a mixture of 2 drops aqua ammonia in 200 cc. distilled water.

Immerse in the ammoniacal silver so diluted as to contain from 2 to 2½ per cent of silver nitrate and keep for 6 days in the dark.

Reduce for from 6 to 12 hours in:

Formol	5.
Pyrogallol	0.5
Water	50.

Embed in paraffin and section.

This method is inconstant and capricious, as are all methods with reduced silver. Its sole advantage and sole indication is that it sometimes brings out clearly the sympathetic neurites of the appendix after picro-formol fixation, which is never accomplished by the technique of Cajal or of Bielschowsky under the same conditions. It does not blacken the neurites (neuroentodermic?) of the mucosa or of the neuromata. It demonstrates the silver-reducing granules but not specifically; for the pyrogallol reduces the silver on the most diverse tissue elements, the nuclei, sometimes the peripheral neuroglia, the various pigments, etc.

This method is of secondary importance; its results should be controlled strictly by my other methods and, in any case, it should

never be trusted alone. I mention it here chiefly to correct an error of Sprafke, to whom I would recommend this method as the method of choice in the study of neurogenic appendicitis.

The photomicrographs are the work of Dr. Charles Simard, to whom I am greatly indebted for his cordial collaboration.

BIBLIOGRAPHY *

Cordier, R. A propos des cellules argentaffines de l'intestin. *Compt. rend. de l'Ass. d. anat.*, Paris, 1921, p. 61.

Cordier, R. Contribution à l'étude de la cellule de Ciaccio-Masson et de la cellule de Paneth, *Compt. rend. Soc. de biol.*, 1923, lxxxviii, 1227.

Cordier, R. Les cellules argentaffines dans les tumeurs intestinales. *Arch. internat. de méd. exper.*, 1924, i, 59.

Cordier, R. A propos de la signification physiologique de la cellule argentaffine. *Compt. rend. Soc. de biol.*, 1925, xcii, 65.

Cordier, R. Recherches morphologiques et expérimentales sur la cellule chromo-argentaffine de l'épithélium intestinal des vertébrés. *Arch. de biol.*, 1926, xxxvi, 427.

Danisch, F. Zur Histogenese der sogenannten Appendixkarzinoide. *Beitr. z. path. anat. u. z. allg. Pathol.*, 1923–24, lxxii, 687.

Ehrlich, S. L. Sur l'origine des carcinoids de l'appendice. Les carcinoides du tractus intestinal et les névromes immatures. Neurocytomes. *Bull. méd. de Kharkow* (in Russian), 1914.

Forbus, W. D. Argentaffine tumors of the appendix and small intestine. *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 130.

Gosset et Masson. Tumeurs endocrines de l'appendice. *Presse méd.*, 1914, xxii, 237.

Hamperl, H. Ueber die "gelben (chromaffin)" Zellen im Epithel des Verdauungstraktes. *Ztschr. f. mikro.-anat. Forschung*, 1925, ii, 506.

Hamperl, H. Ueber die "gelben (chromaffin)" Zellen des Magendarmtraktes. *Verhandl. d. deutsch. path. Gesellsch.*, 1927, 171.

Hamperl, H. Ueber die "gelben (chromaffin)" Zellen im gesunden und kranken Magendarmschlauch. *Virchows Arch. f. path. Anat.*, 1927, cclxvi, 509.

Hasegawa, T. Ueber die Carcinoide des Wurmfortsatzes und des Dünndarmes. *Virchows Arch. f. path. Anat.*, 1923, ccxliv, 8.

Kull, H. Die chromaffinen Zellen des Verdauungstraktes. *Ztschr. f. mikro.-anat. Forschung*, 1925, ii, 163.

Lauche, A. Die Heterotopien des ortsgehörigen Epithels im Bereich des Verdauungskanals. *Virchows Arch. f. path. Anat*, 1924, cclii, 39.

* This bibliography is far from complete; it is limited to the recent writings mentioned in this paper. For the older literature, consult Forbus.

Maresch, R. Ueber das Vorkommen neuromartiger Bildungen in obliterierten Wurmfortsätzen. *Wien. klin. Wchnschr.*, 1921, xxxiv, 181.

Masson, P. La glande endocrine de l'intestin chez l'homme. *Compt. rend. Acad. d. sc.*, 1914, clviii, 59.

Masson, P. Les Névromes sympathiques de l'appendicite oblitérante. *Lyon chir.*, 1921, xviii, 281.

Masson, P. Les lésions nerveuses de l'appendicite chronique. *Compt. rend. Acad. d. sc.*, 1921, clxxiii, 262.

Masson, P. Les lésions nerveuses de l'appendicite, *Congrès de Médicine*, Strasbourg, 1921.

Masson, P. La neurogenèse dans la muqueuse de l'appendice pathologique. Rôle des cellules argentaffines dans ce phénomène. *Compt. rend. de l'Ass. d. anat.*, Gand, 1922, 217.

Masson et Berger. Sur un nouveau mode de sécrétion interne: la Neurocrinie. *Compt. rend. Acad. d. sc.*, 1923, clxxvi, 1748.

Masson, P. Appendicite neurogène et Carcinoides, *Ann. d'Anat. path. médico-chir.*, 1924, i, 3.

Sprafke, H. Untersuchungen über die argentaffinen Zellen in der Schleimhaut des Wurmfortsatzes und ihre Beziehungen zur Entstehung der sogenannten Karzinoide. *Frankfurt Ztschr. f. Path.*, 1927, xxxv, 302.

Schweizer, P. Ueber neuromartige Bildungen in obliterierten Wurmfortsätzen. *Schweiz. med. Wchnschr.*, 1922, iii, 1202.

Van Campenhout, E. Etude sur le développement et la signification morphologique des îlots endocrines du pancréas chez l'embryon de mouton. *Arch. de biol.*, 1925, xxxv, 45.

Van Campenhout, E. Contribution à l'étude de l'histogénèse du pancréas chez quelques mammifères. Les complexes sympathico-insulaires. *Arch. de biol.*, 1927, xxxvii, 121.

DESCRIPTION OF PLATES

PLATE 44

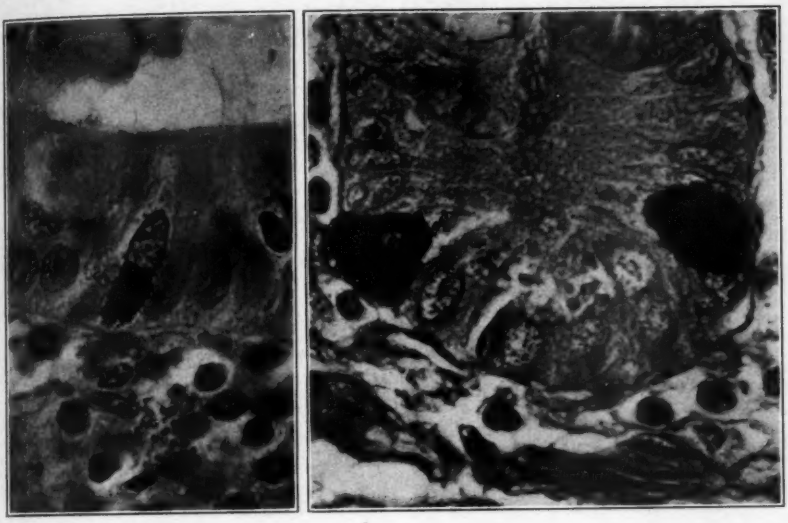
FIG. 1. Chromo-argentaffin cells of the normal intestine.

On the right, section of jejunum after silver treatment in the block. Tip of gland of Lieberkühn with two chromo-argentaffin cells, the granules relatively large and opaque, massed in the basal portion of the cell. The granules conceal the sides of the nucleus; a few are found above the nucleus. The reticulated tissue of the jejunal submucosa contains a few delicate nerve filaments, much fewer than the appendicular mucosa, even normally.

On the left, duodenum. Silvering of the sections. Argentaffin cell in the middle of a gland of Lieberkühn. Tiny uniform granules massed in the basal pole of the cell; a few above the nucleus. Note that they are much smaller in silvered sections than when silvered in the block. × 1100.

FIG. 2. Appendix of male aged 20 years, removed after three slight crises (Obs. 88c). Silvering in the block. Celloidin section.

Argentaffin cells singly and grouped in the subglandular tissue. All the cells are enclosed by nerve filaments (see next Figure). Several chromo-argentaffin cells in the gland epithelium. × 46.



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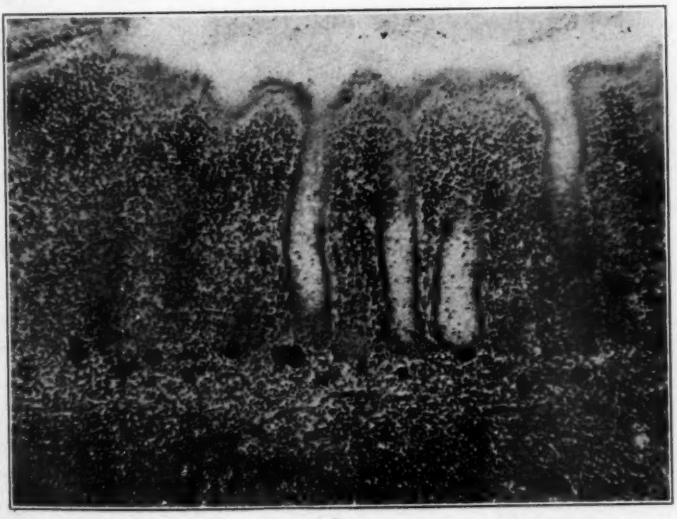

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PLATE 45

FIG. 3. Budding; initial stage (Obs. 88c). Silvering of the section; ponceau-acid fuchsin-aniline blue.

Above and to the left, the bottom of a gland of Lieberkühn. Below and in contact with it nerve filaments of the periglandular plexus. The filaments cut in various planes are surrounded by a collagen sheath, black in the photograph but blue in the preparation. Within this sheath is seen the neuroglia, gray in the Figure, red in the preparation. In cross-sections of the nerve fibers the neuroglia presents a uniformly alveolar appearance. In longitudinal section the alveoli are long, intercommunicating and tubular, running in the direction of the length of the nerve. Inside of these tubes is the pale pink, homogeneous protoplasm of the neurites. At this point there is no hypertrophy of the plexus. Note how much richer it is than in the rest of the intestine (*cf.* Fig. 1). Between the nerves are lacunae of lymphoid tissue. The gland of Lieberkühn is sprouting a bud, not very prominent as yet, formed by a syncytium with four nuclei. The cytoplasm is homogeneous and without granules. × 1100.

FIG. 4. Budding; initial stage (Obs. 88c). Silvering of the sections; ponceau-acid fuchsin-aniline blue.

Here the bud projects directly into a large nerve; the collagen sheath of the nerve is directly continuous with the basement membrane of the gland. There are still no granules in the cells. × 1100.

FIG. 5. Budding more advanced (Obs. 88c). Silvering of the sections; magenta-picrocarmine.

Three nuclei of the bud are surrounded by an undivided cytoplasm in continuity with the lining of the gland. Only the cell which has advanced farthest into the nerve is individualized and granular. Nerve plexus hyperplastic; filaments numerous and broad. Above and to the left, an isolated nerve containing a single argentaffin cell. × 1100.

FIG. 6. Budding more advanced (Obs. 88c).

Multicellular bud in a much enlarged nerve. The four cells which migrated earliest into the nerve are individualized and granular; the others, still attached to the gland epithelium, form a multinucleated syncytial mass. × 1100.

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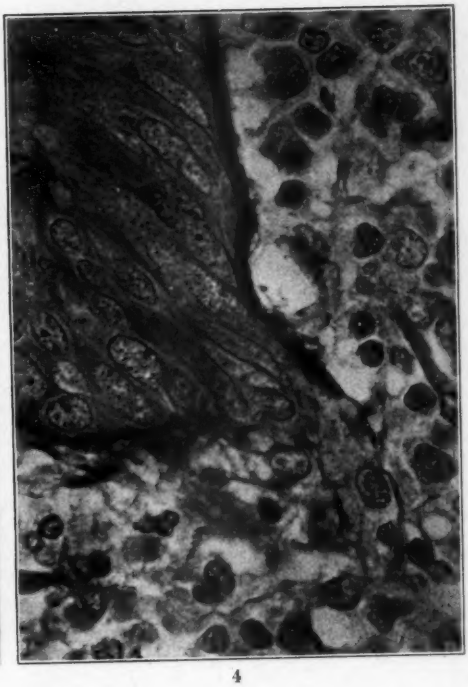

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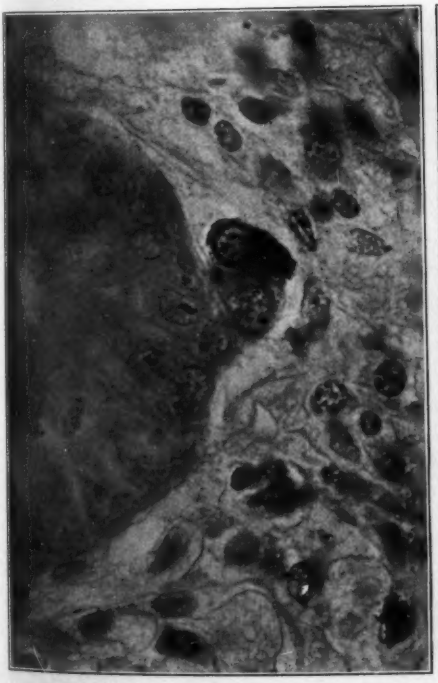

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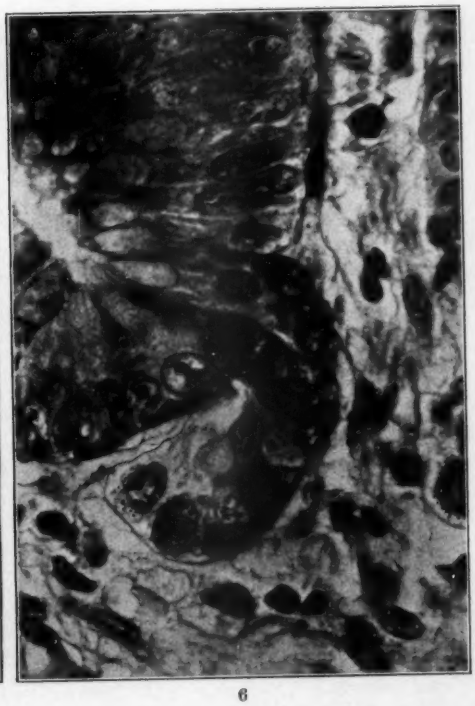

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Masson

Carcinoids (Argentaffin-Cell Tumors)

Plate 46

Fig. 7. Budding more advanced (Obs. 88c). Trichrome.

Swollen bud in a very hyperplastic nerve plexus. The bud is attached to the gland epithelium by a narrow pedicle. Only the two cells farthest advanced into the nerve are individualized and granular. × 1100.

Fig. 8. (Obs. 88c). Trichrome.

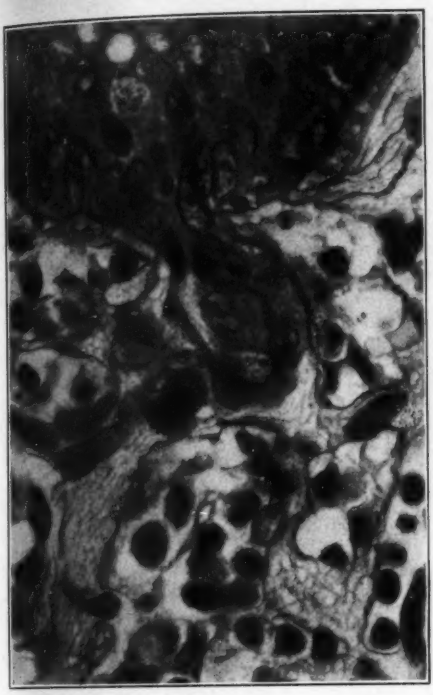
On the right, above and below, tips of glands of Lieberkühn. The left of the Figure represents the subglandular region of the reticulated tissue of the mucosa. At one side of the upper gland is a swollen nerve containing two argentaffin cells. From this same gland hangs a bud with slender pedicle ready to part. The bud is enclosed completely in a nerve bounded by its sheath. At several points the cells of the bud are separated from this sheath by neuroglia of spongy appearance. All cells are granular except those of the pedicle. Lower, argentaffin cells detached from their gland matrix and grouped around a cavity full of colloid (vesicle). These cells are enclosed in a hypertrophied nerve, their cytoplasm is continuous without clear demarcation from the neuroglial cytoplasm. × 1100.

Fig. 9. End of budding; differentiation of the argentaffin cells (Obs. 88c). Silvering of the section; ponceau-acid fuchsin-aniline blue.

Swollen bud in the gland plexus. The pedicle is formed by a large hydropic cell with an elongated nucleus staining homogeneously. Seven argentaffin cells are grouped around a cavity full of colloid. Around them and especially below them are seen elongated cells, their protoplasm containing a few argentaffin granules; they are separated from the rosette and seem to be incorporated in the neuroglia. × 1100.

Fig. 10. Vesicular group detached from the gland epithelium. Differentiation of the cells (Obs. 88c). Trichrome. Colored photograph.

In the center, a vesicle with a small cavity filled with colloid and bordered by tall cells, two of which reproduce exactly the form of the chromoargentaffin cells of the intestinal epithelium. On the right a triangular chromaffin cell having no connection with the cavity of the vesicle. Above the spherical nucleus an angular figure (Nissl body?). Below and on the left cells difficult to identify of ganglionic or neurocrine appearance. Below transition forms between argentaffin and neuroglial cells, elongation of the nuclei, effacing of cellular contours, disappearance of the granules, appearance of intracytoplasmic tubular cavities. × 1800.



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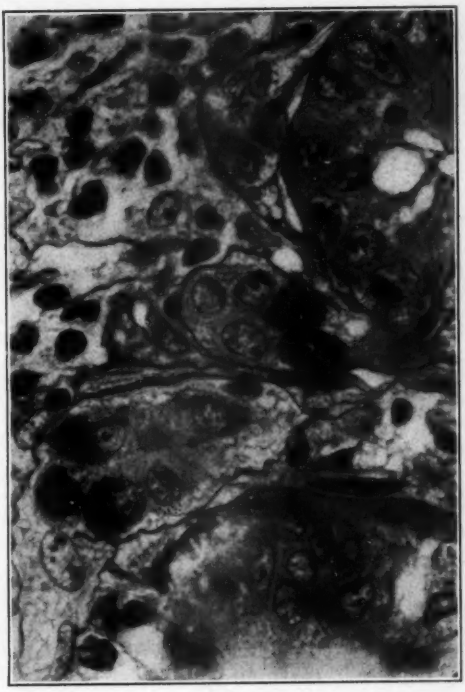

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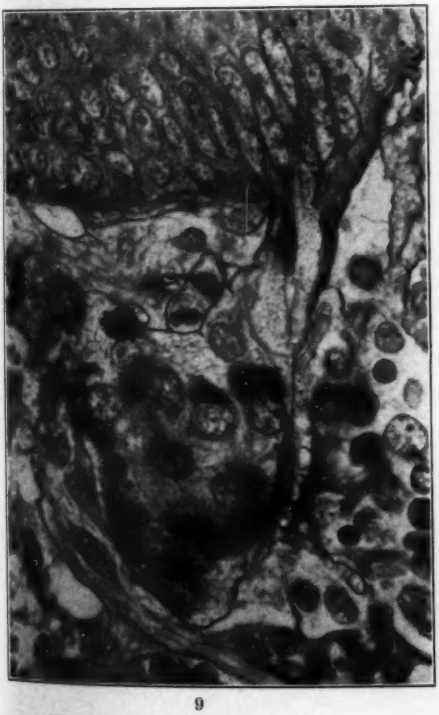

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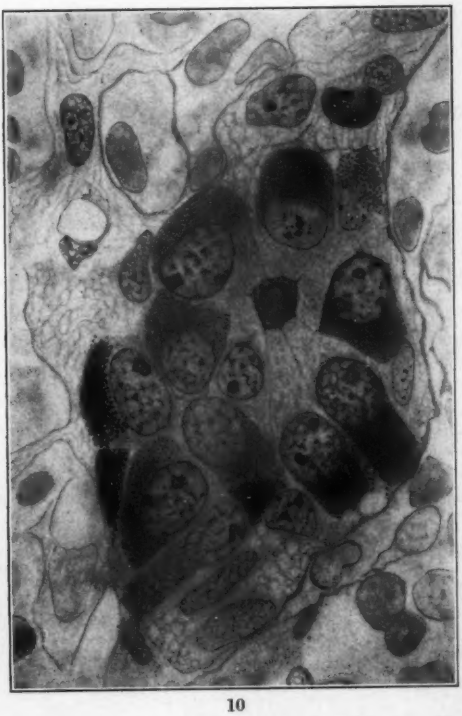

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Masson

Carcinoids (Argentaffin-Cell Tumors)

Plate 47

Fig. 11. Neurocrinia (Obs. 167e). Formol-picric fixation; Marchi's fluid, 8 days. Magenta-picro-indigo-carmine.

Group of neurocrine cells in a hypertrophied nerve. Cells finely granular, contours indistinct, the cytoplasm continuous with that of the neuroglia. In the cytoplasm appear droplets of fat (blacken with osmic acid) which grow larger and escape from the argentaffin cells, being eliminated into the nerve where they soon disappear. They are found only in the immediate vicinity of the argentaffin cells. × 1800.

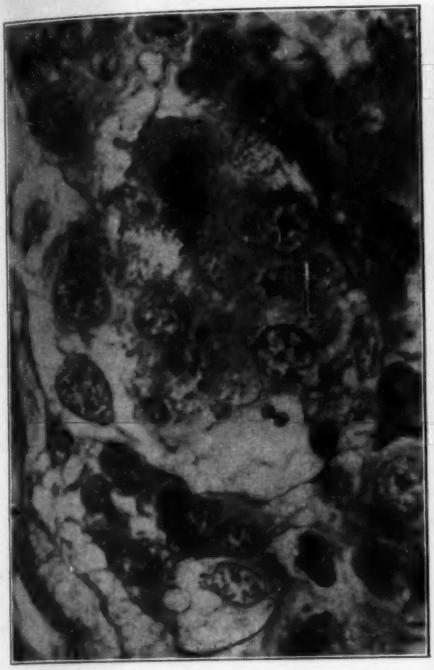
Fig. 12. Vesicle of argentaffin cells isolated in a greatly hypertrophied nerve filament (Obs. 568 ND). × 1100.

Fig. 13. Two argentaffin cells (neurocrine or ganglionic) enclosed in a nerve (Obs. 81c). Impregnation with reduced silver.

On both sides of the cells are seen four neurites which have taken the silver; it is impossible to see their relations with the cells on account of the black granules which fill the cells.

Fig. 14. Ganglion of the intramucous plexus invaded by argentaffin cells (Obs. 88c). Trichrome.

Below and on the right, two non-granular ganglion cells; on the left, two neuroglial nuclei. Above, vesicle of argentaffin cells. In the nerves which leave the upper border of the ganglion, scattered argentaffin cells. In the left upper corner, the edge of a gland of Lieberkühn from which these argentaffin cells have escaped. × 1100.



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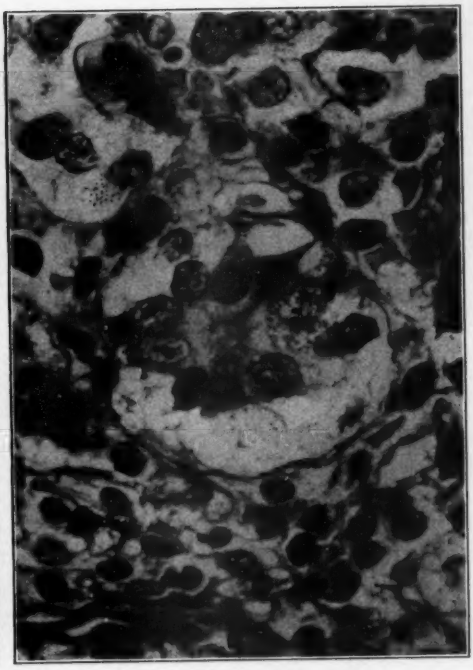

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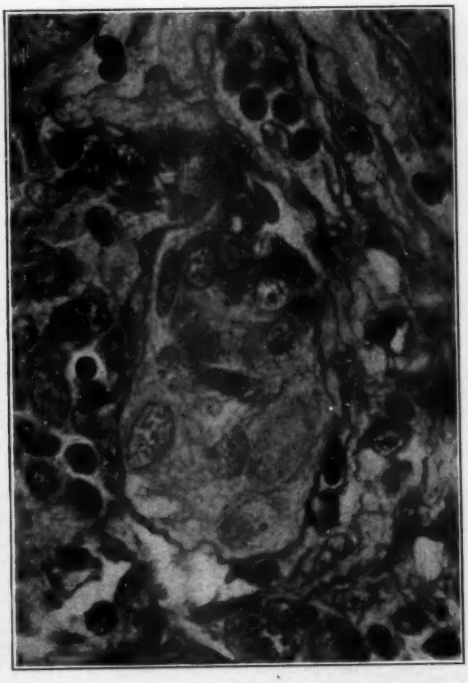

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Carcinoids (Argentaffin-Cell Tumors)

Plate 48

Fig. 15. Connections of certain intestinal cells with the nerves persisting after budding (Obs. 88c). Silvering of the sections. Magenta-picro-indigo-carmine.

On the right and above, tip of a gland (lumen of the appendix on the right). From this tip (on the left of the Figure) hangs an intranervous bud in full activity. Below and on the right, three cells of the gland epithelium are continuous with the neuroglia of the plexus by means of slender prolongations. Near the prolongations, a round argentaffin cell. It is probable that this cell was recently detached from the epithelium at the very point where the cylindrical cells are in continuity with the neuroglia.

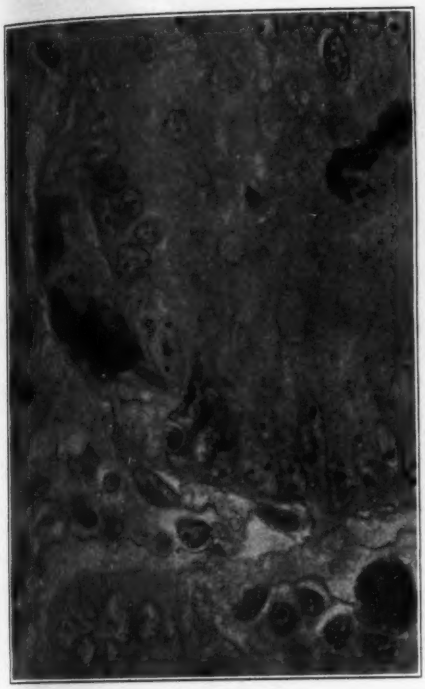
Such views show the actual and direct continuity of certain epithelial cells with the plexus. They show besides that the buds grow from the gland toward the nerve and that the process cannot be an immigration of hitherto intranervous cells into the gland (Danisch). × 1100.

Fig. 16. Neuroma formed in the depth of the mucosa, pushing before it the muscularis mucosae and the submucosa (Obs. 81c). Trichrome. Light area, the neuroma. × 48.

Fig. 17. The deep region of this neuroma.

On the left, in black, the muscularis mucosae. On the right, the neuroma. Above, an argentaffin cell of ganglion type in a node of the neuromatous plexus. × 400.

Fig. 18. Another neuroma from the same specimen, 81c. Impregnation with reduced silver. × 400.



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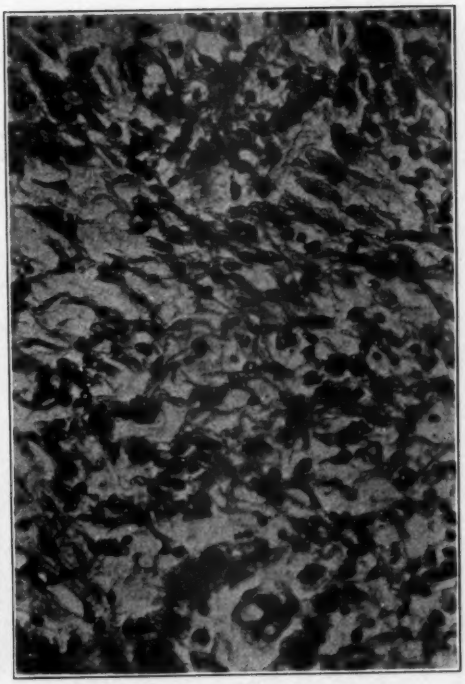

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Masson

Carcinoids (Argentaffin-Cell Tumors)

Plate 49

Fig. 19. Appendix partially stenosed. Laterotерminal neuroma in process of growth (Obs. 228f). Trichrome.

Throughout its permeable portion, the appendix contains tiny intramucous neuromata similar to those of Figs. 16, 17 and 18. At the end of the permeable region, one of these neuromata has grown quite large (light triangle occupying two-thirds of the Figure). This neuroma contains many argentaffin cells. × 48.

Fig. 20. An area in the same neuroma. × 800.

Fig. 21. Appendix completely stenosed (see Fig. 23). (Obs. 773c.) Large axial neuroma. Below and on the right, lymphoid mass representing a neuroma in regression (see Fig. 26). Trichrome. × 48.

Fig. 22. Central region of the same neuroma.

Non-medullated fibers cut across and lengthwise, anastomosing in a compact plexus. × 800.

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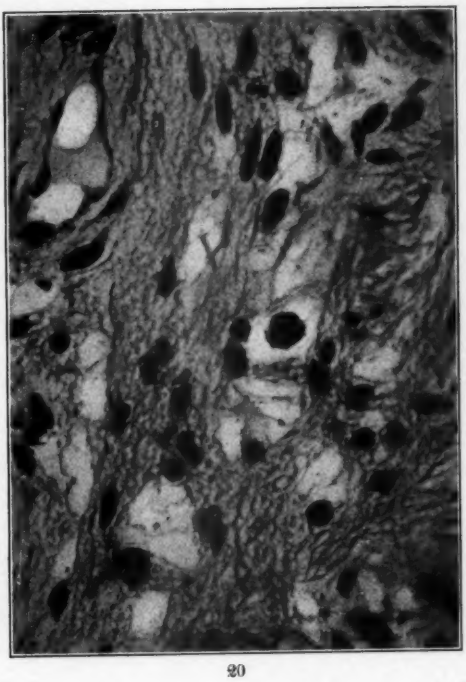

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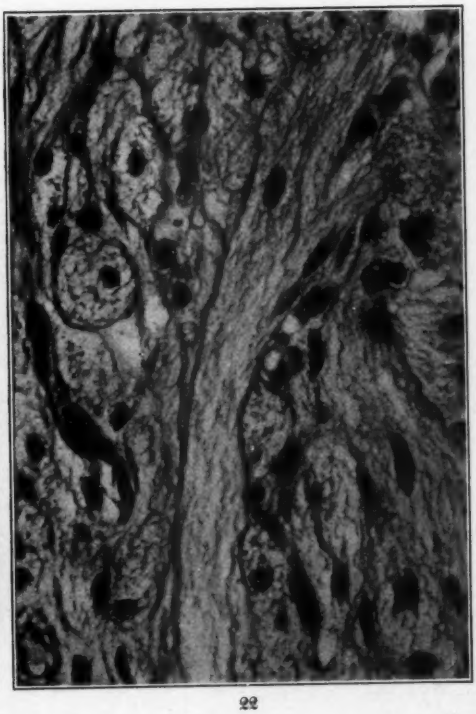

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Masson

Carcinoids (Argentaffin-Cell Tumors)

Plate 50

Fig. 23. Appendix completely stenosed, distal end. Chain of neuromata formed in the axis (Obs. 773c). Trichrome.

Nerve nodules linked transversely and longitudinally by innumerable nerve filaments. In the center of the upper left neuroma, an argentaffin vesicle. The two lymphoid masses represent neuromata without argentaffin cells on the road to resorption. × 100.

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Masson

Carcinoids (Argentaffin-Cell Tumors)

Plate 51

Fig. 24. Argentaffin vesicle (neuro-epithelial rosette?) in the neuroma of Figs. 21 and 22 (Obs. 773c). Trichrome.

The rosette is filled with colloid. Its cells are buried in the neuroglia. The black fibers represent the incomplete collagen sheaths of the nerve fibers anastomosed in an inextricable plexus. × 800.

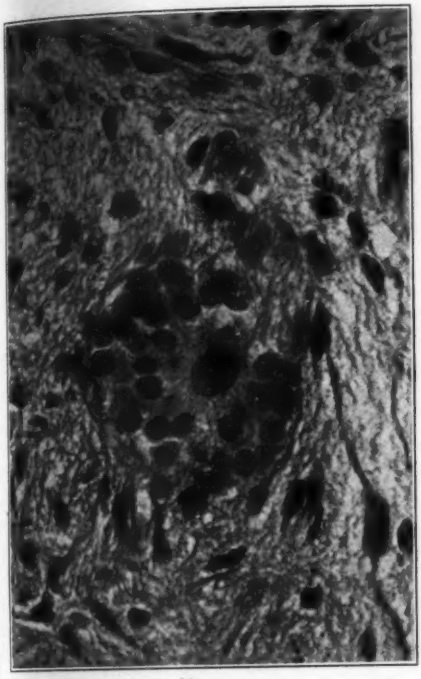
Fig. 25. Longitudinal nerve fibers linking neuroma nodules situated above and below but outside of the Figure (Obs. 69d). Impregnation with reduced silver. Here the silver has stained the neuroglia electively but not the neurites. × 400.

Fig. 26. Degenerating neuroma (Obs. 773 c). Trichrome.

Some fibers recognizable, more or less altered and separated from one another by diffuse lymphoid infiltration. × 800.

Fig. 27. Neurocarcinoid. Trichrome.

Terminal carcinoid from a child 9 years of age. Spherical tumor, 3 mm. in diameter, formed at the stenosed end of the appendix. The margin of the tumor is invading the submucosa; its center is formed by an axial neuroma. Above are seen the plexiform fibers of this neuroma. Below, these fibers are distended with neurocrine cells which are continuous with carcinoid columns, invading the connective tissue of the submucosa. × 800.



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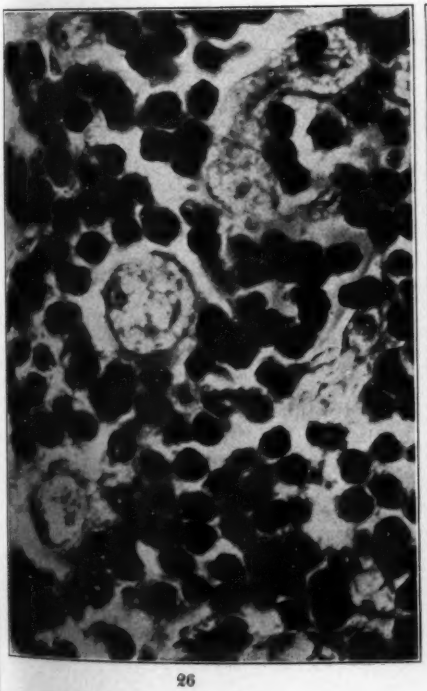

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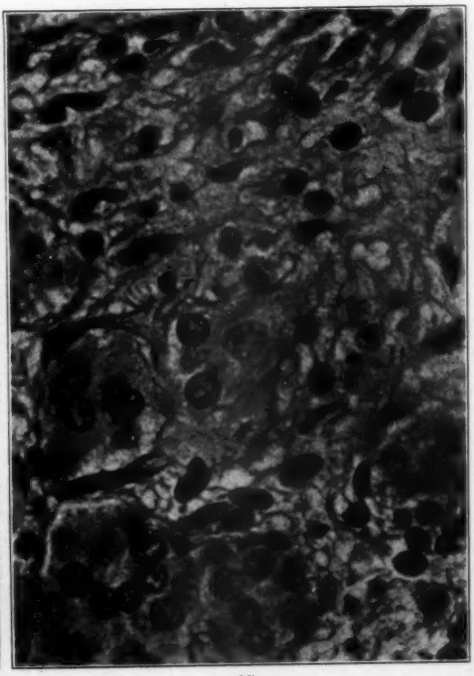

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Masson

Carcinoids (Argentaffin-Cell Tumors)

Plate 52

Fig. 28. Carcinoid cells. Cell column with vesicles, continuous with two columns of palisade cells (Obs. 102f). Trichrome.

Above, vesicle formed by cylindrical cells; none of the cells figured is granular. Kittleisten are seen at the margin of the cavity. Stroma scanty and well vascularized. The long black nuclei in the stroma are nuclei of smooth muscle fibers. × 1100.

Fig. 29. Column formed exclusively by spongy cells (Obs. 102 f). Trichrome.

Resemblance to the adrenal cortex. Note the intimate connections of the spongy cell column with the capillaries (upper right corner). The protoplasm of these cells contains many argentaffin granules. × 1100.

Fig. 30. Thick column formed by spongiocytes and bordered by palisade cells. Vacuoles in their supranuclear region. In the center of the photograph, a racket-shaped palisade cell.

Fig. 31. Vesicles formed by argentaffin cells of intestinal type. Case of Delbet and Herrenschmidt. Silvering of the sections. Picro-indigo-carmine. × 1100.

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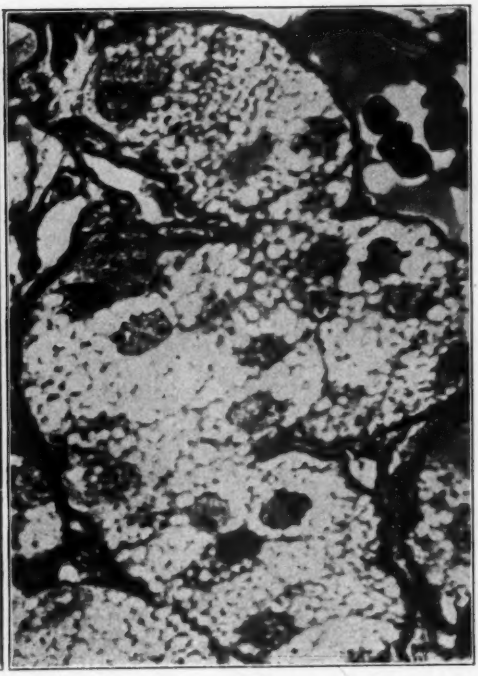

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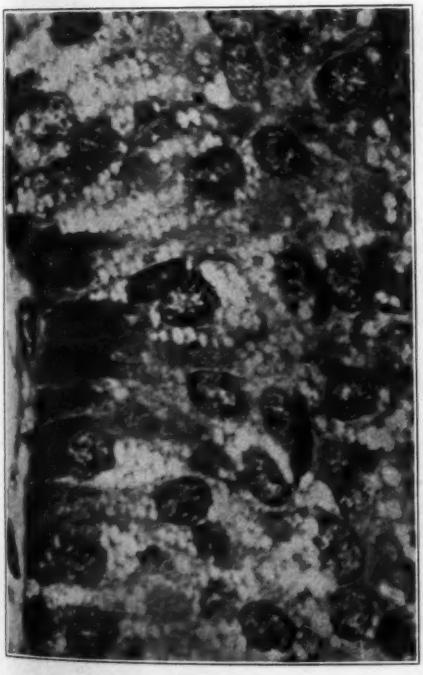

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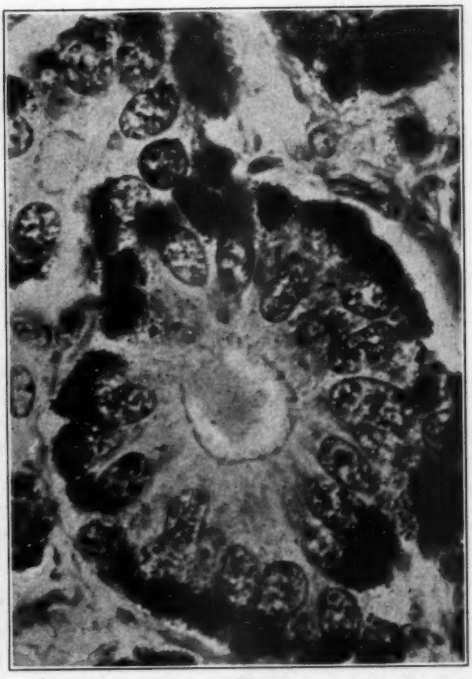

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Masson

Carcinoids (Argentaffin-Cell Tumors)

PLATE 53

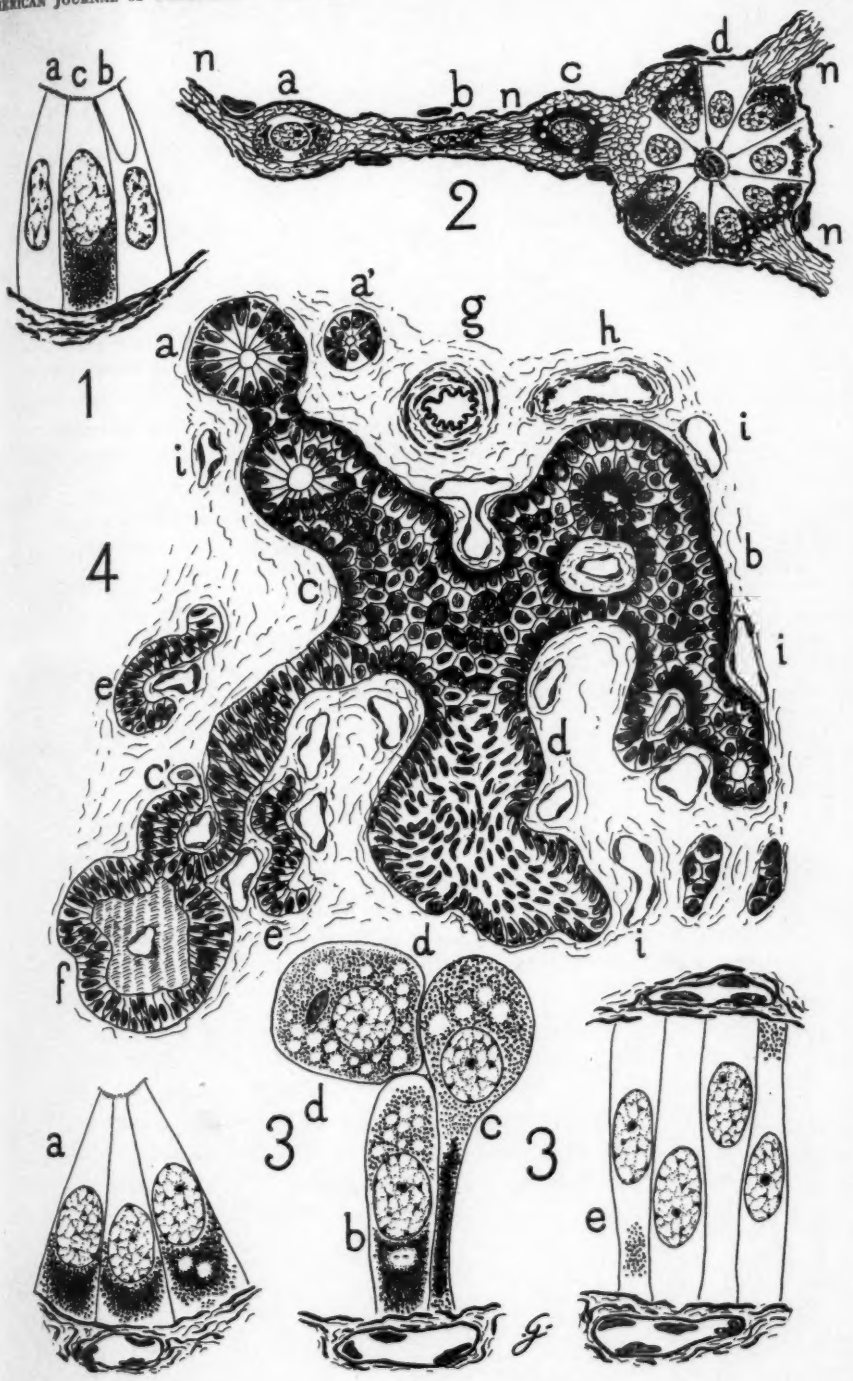
Recapitulation

FIG. 32. 1. Tip of a gland of Lieberkühn: (*a*) "indifferent" cell; (*b*) caliciform cell; (*c*) Kulchitzky cell.

2. Neuroma cells: (*n*) nerve; (*a*) cell of ganglion type; (*b*) cell of neuroglia type; (*c*) neurocrine cell; (*d*) cylindrical cells of intestinal type (Kulchitzky) forming a vesicle.

3. Carcinoid cells: (*a*) cylindrical cells of intestinal type (Kulchitzky) forming vesicles; (*b*) cylindrical palisade cell, the basal region very granular, containing a diplosome, the upper end vacuolated (lipoids) but little granular; (*c*) racket-shaped cell, swelling of the upper end, pointed at the basal end; (*d*) polygonal cell, showing central nucleus, parasome, argentaffin granules distributed diffusely and lipoid vacuoles. When numerous, these vacuoles give to the cell a spongy appearance; (*e*) cells from a pure palisade column, granules few, unevenly distributed, at only one pole of the cell.

4. Continuity of different forms of cell columns in carcinoids: (*a*) column with vesicles of cylindrical cells; (*a'*) column with vesicles of small cuboid cells; (*b*) column of polygonal cells; (*c*) palisade and polygonal cell column; (*c'*) penetration of vessels into the palisade and polygonal cell column, angiotropism; (*d*) column of palisade and fusiform cells; (*e*) pure palisade column (note relation with capillaries); (*f*) mucoid imbibition of the connective tisuse, cylindromatous evolution; (*g*) arteriole; (*h*) venule; (*i*) capillaries.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THE PATHOLOGIC ANATOMY OF TULAREMIA IN MAN *

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In the publication by Francis and Callender,[1] April 1927, of a study of the microscopic changes in tularemic lesions of man three autopsies are recorded. Other than these we have found no record of a post-mortem investigation of the human disease. In two of the recorded autopsies secondary acute infections were present. This, however, did not mask the lesions of tularemia.

The first autopsy was that reported by Dr. J. R. Verbrycke[2] in 1924. The post-mortem examination was made by Dr. Maurice Sellinger upon the body of a patient who died eighteen days after the onset of illness. The lesions were therefore early. The second case, that recorded by Dr. Francis,[3] died twenty-six days after the onset of illness. The third case, examined by Dr. A. J. Bruecken and reported by Francis and Callender, died about five months after a presumptive exposure to the disease. The case reported by Dr. Francis contracted the disease from the bite of a fly; the other two were apparently caused by handling wild rabbits.

In the first case macroscopic lesions attributable to tularemia were found in the lungs, peribronchial lymph nodes and spleen; in the second, lesions were noted only in the spleen; in the third case infected lymph nodes had recently been removed surgically from the left axilla, and at autopsy tularemic lesions were found in the lungs, peribronchial lymph nodes, liver, spleen, and a lymph node beside the cystic duct. No initial cutaneous lesion was described in any of the three cases.

In addition to these autopsies tularemic lesions have been studied in the conjunctiva,[4] in subcutaneous tissue,[5] and in lymph nodes in material removed surgically. Francis and Callender described the lesions in surgical specimens from seven cases.

An initial ulcer of the skin, which is frequently associated with the disease, has not heretofore been described histologically. It was the primary lesion in the case to be reported.

* Received for publication March 26, 1928.

From published studies it is found that the early lesions in the lymph nodes, lungs, spleen and liver are focal areas of necrosis with a cellular reaction consisting of epithelioid cells and polymorphonuclear leucocytes. When these areas undergo caseation, they resemble tubercles very closely. Langerhans' cells have frequently been observed about the margins of the necrotic areas. The lesions tend to be chronic, and healing is indicated by the ingrowth of fibroblasts about the margins, finally encircling the necrotic focus with a fibrous capsule. Suppuration of superficial nodes is described.

Individually the lesions are often not to be distinguished from tubercles. Evidence of their non-tuberculous nature is the absence of tubercle bacilli in stained preparations, and the failure of guinea pigs, inoculated with material which has been subjected to conditions which destroy *B. tularense* but not the tubercle bacillus, to contract tuberculosis.[1] The true nature of the infection in man has in some cases been demonstrated by inoculating rabbits, guinea pigs or white mice, which are very susceptible to tularemia, with infectious material, and by the appearance in the patient's blood of specific agglutinins ten days to two weeks after the onset. The *Bacterium tularense* has never been demonstrated in smears or sections of human tissue. Nor is it always demonstrable in similar lesions of lower animals. The microörganism, however, may be readily cultivated from suitable material by the use of proper media.

There are many ways by which the disease may be transmitted from an infected host to man. By far the greatest number of such transmissions follow the handling of infected rabbits, and begin with an indolent ulcer which develops at the site of an abrasion on the hand. Primary opthalmic tularemia may also follow similar exposures. Other modes of transmission are by the bite of the wood tick and the deer fly (*Chrysops discalis*), an ulcer developing at the site of the bite. The disease has been known to occur, following the handling of infected rabbits, without the development of a local lesion. Ohara [6] proved by human inoculation that such could occur. There is a typhoid form of the disease, the type usually presented by laboratory workers who contract it from handling infected animals. In this form there is usually no local lesion. Droplet infection [7] may play a rôle in some of these case.

Whether or not specific agglutinins invariably appear has not

been proved, but it is assured that they may be absent in early stages of very acute fulminating forms of the disease.

In human infections a history of a wound, received while handling wild rabbits, followed by an indolent ulcer on the hand at the site of the injury, inflammation of lymph nodes draining this area, and a febrile reaction, offer good presumptive evidence of a tularemic infection. A positive aggultination of *B. tularense* by the patient's serum serves to confirm the diagnosis.

The mortality from the disease is relatively low, namely, about three per cent.

During the past fall and early winter thirty-one cases of tularemia have been reported in Davidson County, Tennessee. A considerable number of cases have been observed also in neighboring counties, and all of these, so far as we have been able to determine, have been due to contact with wild rabbits. Only one fatality has come to our attention. This was a typical case of the ulcero-glandular type which ran a violent course terminating in death after fifteen days. An autopsy was performed within an hour after death, and the study of this material is the basis of our report.

Report of Case

Clinical Report: A. T. R., a white, male, American, aged 29 years, presented himself for examination on December 1, 1927, complaining of a painful swelling in the right axilla, general malaise, and a sore finger on the right hand.

Past Illnesses: On September 26, 1927, the patient became ill with typhoid fever. He had fever for about three weeks and made a fairly satisfactory convalescence. He left the hospital, under protest, on October 20, 1927, about one week after being afebrile.

Occupation: About November 15, 1927, he secured a position with a produce house, dressing fowl and rabbits.

Present History: On November 26, 1927 while dressing rabbits the right index finger was scratched. On November 28, while dressing rabbits the right index finger was injured again with a rabbit bone. On November 29, there was a small, painful swelling of the injured finger. On November 30 soreness was noted in the right axilla. On December 1, as stated, the patient presented himself for examination.

Physical Examination: There is slight evident pallor; temperature 101° F; pulse 100; respiration 20. There is an area of redness and induration on the dorsum of the terminal phalanx of the right index finger, more marked around the nail. There is a small (2 mm.) crust-covered abrasion in the center of the indurated area. There are no evident red streaks and no glands or tender areas palpable along the arm. There is a very tender mass in the right axilla which on careful examination appears to be an agglutinated mass of enlarged glands. The

entire mass is estimated to be about 8 cm. in diameter. There is a palpable gland in the right supraclavicular fossa about 2 cm. in diameter, and not tender; no other superficial glands are enlarged or tender. The examination is otherwise essentially negative. The leucocyte count is 13,000. On account of the history and findings, a working diagnosis of tularemia was made.

Course: Treatment was carried out at home for two days. Hot wet packs were applied to the finger continuously, and dry heat to the axilla. Aspirin was given q. 4 h. In spite of rather energetic treatment along these lines the patient's fever rose to 103° F, the glands in the axilla continued to swell and the general condition of the patient became progressively worse. He was sent to the hospital on December 3, 1927. At this time about a drop of pus had accumulated at the site of the abrasion in the center of the area of induration; and this was evacuated. On admission to the hospital the leucocyte count was 15,000; the red cell count 3,750,000; the hemoglobin 75 per cent. The differential count showed 72 per cent polymorphonuclear leucocytes, 16 per cent small lymphocytes, 6 per cent large lymphocytes, 5 per cent mononuclears, and 1 per cent eosinophils. Two days later the leucocyte count was 19,000; the urine was negative. A few hours after admission nausea and vomiting occurred and continued throughout the illness. The patient complained of pain in the head, throat, neck, back, joints, and abdomen. The teeth became very sore, and there was an extremely bad taste in the mouth. On December 4, the temperature and pulse returned to normal for a few hours during which time the patient seemed better. The following day the temperature reached 104° F. On December 9, the mass of glands in the right axilla was only about 3 cm. in diameter, and not very tender. On this day the temperature again came to normal for a few hours without any apparent improvement in the patient's condition, and there was more pain than usual in the abdomen. Examination revealed marked rigidity and tenderness in the right upper quadrant. It was thought that much of the rigidity was due to an enlarged liver, the lower edge of which was not palpable. On the following day, however, it was felt with more certainty that the liver was greatly enlarged. The spleen also became palpable on this day and was very firm. Treatment necessarily remained symptomatic, and this was of little avail. The patient became progressively weaker. On the night of December 11, twelve days after the appearance of the lesion on the finger, blood was drawn and kept in the ice-box over night. The serum from this sample was divided and half of it was sent to Dr. Francis at Washington, who reported that it did not agglutinate *B. tularense* (this serum was inactivated before being mailed). The other half, without being inactivated, was sent to Dr. R. L. Jones, of the City Health Department, who reported positive agglutination in dilutions of 1:40 and 1:80 with *B. tularense.*

On December 13, the patient's condition was most critical, it being evident that the end was very near. A transfusion of 300 cc. of citrated blood was introduced into the patient's vein. Two and one-half hours after transfusion he died. The patient lived fifteen days after receiving the second injury on his finger. A few hours before death the leucocyte count had risen to 22,600.

Pathologic Report

The body was that of a young white man, well developed and well nourished. It was still warm and rigor mortis had not set in. The

head, eyes, ears, nose and mouth presented no noteworthy abnormalities. The thyroid gland was not enlarged. There was no edema.

On the dorsal surface of the terminal phalanx of the right index finger next to the nail there was a circular ulcer 8 mm. in diameter. The base was about 1 mm. deep. It had a brownish, opaque, ne- [illegible] little evidence of inflammation about

ERRATUM

In the paper on the Pathological Anatomy of Tularemia in Man by Goodpasture and House, appearing in the American Journal of Pathology, Vol. IV, No. 3, May, 1928, on page 216, the statement is made that a specimen of serum removed on December 11, 1927, *twelve days* after onset of symptoms of tularemia, was sent to Doctor Francis at Washington and to Doctor R. L. Jones of the City Health Department at Nashville, Tennessee, for agglutination reaction with *B. tularense*. It is stated in our report that Doctor Francis found this specimen negative and that it was found positive by Doctor Jones in dilutions of 1:40 to 1:80. This statement is incorrect. A specimen of serum from this case was sent to Doctor Francis on December 3, 1927, *four days* after the onset of symptoms and was found by him to be entirely negative in agglutination tests with *B. tularense*. Another specimen removed on December 11, 1927, *twelve days* after the onset of symptoms, was sent to Doctor Jones, who reported positive agglutination with *B. tularense* in dilutions of 1:40 to 1:80.

(Signed) E. W. GOODPASTURE and S. JOHN HOUSE.

[illegible]

Pericardial Cavity: There was a normal amount of clear, straw-colored fluid in the pericardial sac. The pericardial surfaces were smooth, shiny and normal in appearance. The heart did not appear to be enlarged, or grossly abnormal.

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Pathologic Report

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On the dorsal surface of the terminal phalanx of the right index finger next to the nail there was a circular ulcer 8 mm. in diameter. The base was about 1 mm. deep. It had a brownish, opaque, necrotic surface. There was very little evidence of inflammation about the margins. The ulcer was excised for histologic examination.

The axillary lymph nodes on the right were easily palpable and distinctly enlarged. A supraclavicular node on the right was palpable. The nodes in the left axilla were not felt, and there was no enlargement of the inguinal nodes.

Peritoneal Cavity: On primary incision subcutaneous fat was presented in moderate amounts. The musculature of the abdominal wall was well developed and of reddish color. The peritoneal surfaces presented a smooth, shiny, moist appearance. There was only a slight increase of peritoneal fluid. The abdominal organs were normally situated. The liver was very much enlarged and extended two fingers breadth below the costal margin in the right mamillary line. The liver edge was firm and rounded. The spleen also was considerably enlarged. The appendix appeared normal. The omentum was fatty and free of adhesions. The stomach was contracted. Greatly enlarged lymph nodes were observed along its lesser curvature and extending into the hilum of the liver.

Thoracic Cavity: On removal of the sternum the lungs were observed to be moderately voluminous and pink. The anterior mediastinum was fatty and contained several enlarged lymph nodes. Enlarged nodes extended from the mediastinum upward into the deep tissues of the neck on either side.

Pleural Cavities: There were about 200 cc. of clear fluid in the left pleural cavity. On the surface of the right pleura, where it adhered to the superior mediastinum mesially, there was a patch of pinkish gray granulations, evidently a localized fibrinous pleuritis adjacent to the mediastinum. A great mass of lymph nodes could be felt about the bifurcation of the trachea, and in the hilum of each lung.

Pericardial Cavity: There was a normal amount of clear, straw-colored fluid in the pericardial sac. The pericardial surfaces were smooth, shiny and normal in appearance. The heart did not appear to be enlarged, or grossly abnormal.

Heart: Weight 350 gm. The valves, endocardium, and coronary arteries presented no noteworthy abnormality. The myocardium was somewhat pale and more opaque than normal.

Lungs: The lungs were everywhere air-containing, except for a superficial zone in the left lower lobe posteriorly where there was atelectasis. There was no evidence of consolidation or inflammation in either lung. The bronchial mucous membranes were pink and normal in appearance. There was, however, a large mass of firm lymph nodes about the bronchi and in the hilum of each lung, and a chain of similar enlarged edematous nodes extended along the esophagus and below the diaphragm. These will be described more in detail below.

Gastro-Intestinal Tract: The stomach was practically empty. The mucosa of the esophagus, stomach, and the small and large intestine throughout presented a normal appearance. Bile could be expressed through the ampulla of Vater with a little pressure on the gall-bladder. The chain of lymph nodes along the lesser curvature of the stomach extending into the hilum of the liver was greatly enlarged. There was a similar enlargement of the periaortic nodes of the upper abdomen. The mesenteric and lower abdominal nodes were not enlarged.

Liver: The liver weighed 3100 gm. It was very large, smooth and rounded. It had a yellowish, mottled appearance. The individual lobules appeared enlarged, and on section here and there tiny gray dots like miliary tubercles could be seen by careful scrutiny. The consistency was fairly firm. The gall-bladder was distended with bile. The lymph node beside the cystic duct was conspicuously enlarged, and in the hilum there was a mass of very large nodes, to be described in detail below.

Pancreas: The pancreas was of normal size, color and consistency. Above and about it were numerous enlarged lymph nodes.

Spleen: The spleen was greatly enlarged weighing 950 gm. It had a smooth surface, a fairly firm consistency and a dark purple color. Through the stretched capsule could be seen several light gray areas measuring up to 1 cm. in diameter. On section the cut surface presented a unique appearance. Very numerous chalky white, or yellowish opaque spots measuring up to 1 cm. produced a striking effect. The larger were irregular in outline almost angular. Their clean-cut margins and their opaque yellowish or whitish color

contrasted sharply with the dark purple background of the pulp. From the centers of the larger areas a soft pasty material could be scraped out with the knife. The Malpighian bodies were not conspicuous. The areas of necrosis in the spleen do not resemble those of tuberculosis, and the effect they produce is the most characteristic feature of the gross pathology of tularemia. The spleen measured 17.5 by 11.5 by 6 cm.

Kidney: The right kidney weighed 180 gm.; the left 170 gm. The capsule of each stripped easily, leaving a smooth, pale, slightly opaque surface. On section the consistency was normal. The cortex presented normal striations. The cortical zone was thicker than normal, and the slight grayish opacity of cloudy swelling was observed. The pelves and ureters appeared normal.

Adrenals: These glands were of normal size and consistency. On section they presented normal markings.

Pelvic Organs: The urinary bladder and prostate presented no noteworthy alterations.

Testes: The left testis appeared normal. The right testis was small, firm and fibrous, having on section a yellowish, opaque appearance, as if partially necrotic and fibrosed.

Brain: The central nervous system was not examined.

Bone Marrow: The marrow of the ribs and right femur was abundant and pink.

Lymph Nodes: The group of lymph nodes in the right axilla draining directly the ulcer of the right index finger were removed. Several of the nodes were considerably enlarged measuring up to 1.5 cm. It was stated that from clinical examination they had diminished in size following great initial swelling. They were discrete and fairly firm. On section the cut surface presented numerous yellowish, opaque spots about 1 mm. in diameter, uniform in size, and regularly distributed throughout the nodes. Beneath the capsule were streaks of a similar, opaque, yellowish color corresponding to the peripheral lymph sinuses. The content of these areas was pasty, but not fluid or purulent. The necrotic material had the appearance and consistency rather of caseous matter, and altogether the process had a considerable similarity to a tuberculous lesion. Each of the enlarged glands from the axilla presented a similar appearance. An enlarged supraclavicular node showed no areas of necrosis on section.

Lymph nodes from the anterior mediastinum and the deep tissues of the neck on both sides presented an appearance similar to that in the nodes from the right axilla. The nodes at the hilum of the lung were bound together by an intervening rather dense fibrous tissue. On section through these masses, the discrete outlines of anthracotic nodes were readily discernible, and their cut surfaces presented likewise the discrete opaque caseous spots. The mass of hilic glands measured 7 by 6 by 4 cm.

The nodes along the esophagus and aorta were discrete and greatly enlarged. On section they were somewhat softer and more edematous than those above. The cut surfaces were mottled by grayish and yellowish areas of necrosis which were larger, more extensive and less of a caseous appearance and consistency than those in the right axillary nodes. These areas had the appearance of a more recent coagulative necrosis, less well circumscribed and containing and being surrounded by a more fluid exudate. This was true also of the nodes along the lesser curvature of the stomach, pancreas and hepatic hilum. In these nodes, which measured up to 4 cm., the necrosis appeared most recent and extensive. All of these nodes were discrete.

Remarks

The infection evidently is preëminently a lymph borne one, extending from the index finger to the right axillary nodes, and thence to the mediastinum, peribronchial and hilic regions, thence upward into the neck and downward along the esophageal and aortic nodes to the upper abdomen, and across through the gastric nodes into the hilum of the liver. Hemal distribution is indicated only by the areas of necrosis in the spleen and the miliary foci in the liver. Grossly the lesions individually in the axillary nodes are indistinguishable from tuberculous caseation. Their uniformity in size, age, and distribution in these nodes is not like tuberculosis, nor is the more recent necrosis of the abdominal nodes. The distribution along the lymphatic chains as presented in this case is again unlike tuberculosis. Also the lesions in the spleen with their chalky appearance, their irregular outline and absence of encapsulation present a picture which is to be regarded as certainly unusual for tuberculosis. Thus, while the lesions individually are very similar to tuberculosis, the pathologic process as a whole has in this case at least a certain distinctiveness.

Histologic Report

Heart: There is a marked congestion and interstitial edema of the myocardium of the left ventricle. Cloudy swelling is present. About some of the veins there is a moderate accumulation of large mononuclear cells.

Lung: A section through a primary bronchus, with a small lymph node attached, shows a small hyalinized healed tubercle in the node.

Aside from areas of atelectasis associated with edema in certain portions, the sections of lung present no noteworthy changes.

The pleura from the upper mesial surface of the right side which showed a granulating appearance in gross, presents a subacute inflammation. The surface is covered with fibrin, within and below which are polymorphonuclear leucocytes and epithelioid cells. Beyond these are numerous lymphocytes.

Thymus: A section of thymus shows thin strands of thymic tissue embedded in adipose tissue. No abnormality is noted.

Gastro-Intestinal Tract: Sections through the stomach show a slight polymorphonuclear cell exudate in the mucosa.

Liver: There are numerous focal scars in the liver. Most of them are about the size of an hepatic lobule and fairly definitely circumscribed. Some of these scars in addition to fibrous strands contain numerous large and small mononuclear cells and an occasional isolated hepatic cell. Others are composed almost compeletely of hyalinized fibrous tissue. An occasional scarred area is quite extensive involving several adjacent hepatic lobules. There is some, but relatively little, regeneration of bile ducts. These scars have the size and distribution of typhoidal lesions, and are considered to be healed areas of focal necrosis.

In addition to the scars there are acute focal lesions undoubtedly of tularemic origin. These are relatively few in number and are irregularly disposed through the hepatic parenchyma. They are of the size and general appearance of small and large miliary tubercles. The earlier ones consist of a focal collection of polymorphonuclear and large mononuclear cells, with little necrosis in the center of the group. Larger ones show central necrosis and caseation with a marginal zone of epithelioid cells. No Langerhans' cells are present.

The hepatic cells are swollen and show the distinct granulation of cloudy swelling. There is considerable congestion.

Pancreas: The pancreas presents no noteworthy abnormality.

Spleen: The splenic pulp is engorged with blood, and much of the blood appears to be extravasated. Great numbers of polymorphonuclear and large mononuclear cells are present, diffusely scattered throughout the pulp. The Malpighian bodies are rather pale and diffuse. Here and there are focal scars, and there is an increase in connective tissue about some of the larger blood vessels. The focal scarring suggests the results of typhoidal necrosis.

Dispersed irregularly through the pulp are large and small areas of necrosis which are evidently of tularemic origin. These areas have necrotic, sometimes caseous centers, with a marginal zone of epithelioid cells. No Langerhans' cells are present. In the center of very large areas of necrosis there is autolysis and partial liquifaction. In smaller areas in which necrosis has not advanced, there are a great number of polymorphonuclear as well as epithelioid cells; and fibrin strands are to be seen.

Adrenals: The adrenals show no noteworthy pathologic changes.

Kidney: The kidneys are congested, and there is conspicuous parenchymatous degeneration.

Urinary Bladder: Sections show no noteworthy pathologic changes.

Testes: One section shows complete atrophy of the seminiferous tubules. There is also hyalinization and atheromatous degeneration of arterial walls. Leidig's cells are not prominent. Another section shows a large infarct, with hyalinization of the infarcted area. Hematoidin crystals are abundant.

Prostate: Sections show no noteworthy pathologic changes.

Lymph Nodes: Sections through the right axillary lymph nodes, which drained the ulcer on the finger, show a subsidence of the acute inflammation. The lymph sinuses are not markedly distended and contain relatively few large mononuclear leucocytes. Corresponding to the opaque yellow spots noted in gross, there are circumscribed areas of necrosis with central caseation and a well developed marginal zone of epithelioid cells. No Langerhans' cells are present. Except for the absence of giant cells these lesions closely resemble tubercles. Here and there a blood vessel in the neighborhood of a caseous focus shows the deposition of thrombus on its intimal surface next to the necrotic area. The thrombus is composed for the most part of fibrin and large mononuclear cells.

Sections through lymph nodes from about the bronchi, esophagus, aorta, stomach, pancreas and hilum of the liver all contain numerous similar lesions but evidently more recent, in that none shows quite so advanced caseation. Various stages in the formation of these lesions may be seen. The lymph node as a whole is edematous and inflamed. The blood vessels throughout are conspicuously dilated and engorged with red blood cells. The lymph sinuses likewise are dilated, containing the granular precipitate of coagulated lymph, numerous large phagocytic cells, polymorphonuclear leucocytes and occasional fibrin strands. Many focal lesions can be seen to originate within a lymphatic space. A network of fibrin is precipitated and in its meshes are the irregularly shaped nuclei of epithelioid cells often associated with numerous polymorphonuclear leucocytes. Other lesions seem to originate in the parenchyma. Here again there is a deposit of fibrin and often large numbers of polymorphonuclear leucocytes are present. The cellular exudate of the foci usually consists of both polymorphonuclear and epithelioid cells, sometimes one and sometimes the other predominating. As the lesion advances necrosis occurs in the center and a marginal zone of epithelioid cells becomes apparent. No Langerhans' cells have been seen in any section, and no lesions older than those in the right axilla have been observed. All of the lesions therefore are acute and necrotizing.

No microörganisms have been demonstrated.

Ulcer of the right index finger (initial lesion): Sections through the center of the ulcer show the base to consist of necrotic tissue which extends deeply. The type of necrosis resembles that seen in the centers of the lymphatic foci, that is, it is a coagulation necrosis resembling caseation. It consists of a pinkish amorphous material containing dust-like particles of fragmented nuclei. In some places nuclei are undergoing karyolysis. About the margins of the necrotic mass there is a zone of polymorphonuclear and epithelioid cells, the one or the other type predominating in different areas. The necrosis extends into the surrounding tissues particularly about arteries, which it tends to surround, leaving the vessel relatively unimpaired in the center. Further out there is a large mononuclear cell and lymphocytic exudate about blood vessels and sweat glands. Sometimes the large phagocytes contain numerous lymphocytes. There is little or no evidence of an acute inflammation in the tissue surrounding the ulcer.

Note: Tissues were fixed in Zenker's solution, Schaudinn's fluid, Helly's fluid and formaldehyde. Paraffin sections were stained, in addition to the routine methods, by Giemsa's, Gram's, the carbol-fuchsin, and carbol-anilin-fuchsin methods for bacteria. No micro-organisms of any kind were revealed. Smears from the fresh lesions similarly stained showed no microörganisms.

Discussion

The diagnosis of this case rests finally upon the history, clinical course, the pathologic anatomy, and a positive agglutinin test. Rapidly following a scratch on the right index finger, received while dressing wild rabbits, a local ulcer developed. The ulcer expanded slowly and its base became lined by necrotic tissue. There was a relatively slight inflammation about it. The subsequent lymph node involvement is directly attributable to the lesion on the finger. First in order the right axillary nodes became enlarged and painful. There was a progressive infection of lymph nodes extending from the right axilla to the mediastinum, downward along the esophagus through the diaphragm and across the lesser curvature of the stomach into the nodes of the hilum of the liver. Microscopically, the ulcer of the finger presents the same type of necrotizing injury and cellular reaction that is found in the lymph nodes, that is, a cellular reaction consisting of polymorphonuclear leucocytes and epithelioid cells aggregated in masses often in a meshwork of fibrin. The focus undergoes a caseating necrosis. The necrosis is surrounded by a marginal zone of epithelioid cells. Many of the foci in lymph nodes begin within lymphatic spaces, others seem to arise within the parenchyma. The entire node becomes acutely inflamed. Blood vessels are dilated. The tissue is edematous and the lymph sinuses are distended with fluid containing numerous cells, large phagocytes, lymphocytes and polymorphonuclear leucocytes intermingling. The larger phagocytes often contain other cells within their cytoplasm. Thus the infection may obviously spread along lymphatic channels. Through these channels the virus may enter the blood stream. Or the blood stream may be entered directly. Frequently one sees in sections of the lymph nodes an area of necrosis encroaching upon a blood vessel which becomes thrombosed. Sometimes necrotic blood vessels are seen in the center of a focus of necrosis.

The bacteria circulate in the blood at intervals if not continuously and this is made manifest by the occurrence of typical foci of necrosis in the spleen and liver. Grossly the appearance of the spleen seems quite characteristic. It is enlarged, distended with blood, and is speckled on the cut surface with opaque yellowish or chalky areas measuring up to about 1 cm. in diameter, sharply circumscribed, irregular in outline and even with the surface. The larger areas have softened centers.

Clinically the right axillary nodes diminished appreciably in size during the course of the disease, and histologically the general exudative reaction is less marked and the foci of necrosis appear older, that is more compact and caseous, than in the lesions elsewhere.

The serum removed on the 13th day of the disease was reported as agglutinating *Bacterium tularense* in one laboratory and was negative in another.

Our case showed no evidences of active infection other than those of tularemia. The pathologic anatomy is regarded as characteristic. The infection as illustrated by this example is essentially lymphatic in distribution resembling in this respect bubonic plague, which the disease simulates in rodents.[8]

It is noteworthy that this case died of what appeared to be an intoxication from a pure tularemic infection. No tularemic or other lesions were found in the lungs. Histologically the lesions differ from most of those previously described only in their recentness and in the absence of Langerhans' cells.

Summary

1. The pathologic anatomy of a typical case of ulcero-glandular tularemia studied by autopsy is presented.

2. The histology of the initial ulcer is described for the first time in the human disease.

3. The lesions are essentially lymphatic in distribution affecting many contiguous groups of lymph nodes, the spleen and liver.

4. The individual lesions have a certain similarity to those of tuberculosis, but collectively they constitute a characteristic picture.

REFERENCES

1. Francis, Edward, and Callender, G. R. Tularemia; microscopic changes of lesions in man. *Arch. Path. & Lab. Med.*, 1927, iii, 577.
2. Verbrycke, J. R., Jr. Tularemia; with report of a fatal case simulating cholangeitis, with post-mortem report. *J. A. M. A.*, 1924, lxxxii, 1577.
3. Francis, Edward. Tularemia. Kolle and Wassermann, Handbuch der pathogenen Mikroorganismen, 1927, Ed. 3, vol. 6. (Cited by Francis and Callender, Ref. 1.)
4. Sattler, Robert. Acute (Bacillus Tularense) Conjunctivitis, *Arch. Ophth.*, 1915, xliv, 265.
5. Permar, H. H., and Weil, G. C. The histopathology of the subcutaneous lesions in tularemia in man. *Am. J. Path.*, 1926, ii, 263.
6. Ohara, H. Experimental inoculation of the disease of wild rabbits into human body, and its bacteriological study. *Japan Med. World*, 1926, vi, 299.
7. Parker, R. R., and Spencer, R. R. Six additional cases of laboratory infection of tularemia in man. *Pub. Health Rep., Wash.*, 1926, xli, 1341.
8. Councilman, W. T., and Strong, R. P. Plague-like infections in rodents. *Tr. Assn. Am. Phys.*, 1921, xxxvi, 135.

DESCRIPTION OF PLATES

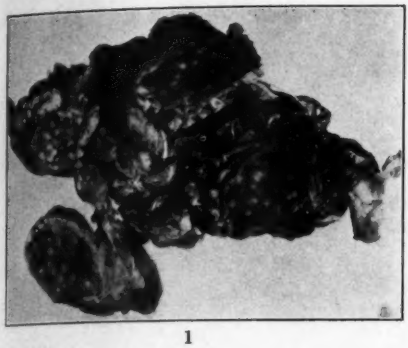
PLATE 54

FIG. 1. Right axillary lymph nodes. The cut surface shows the discrete gray spots of necrosis. Actual length of specimen 5 cm.

FIG. 2. Section through a mass of peribronchial lymph nodes. Actual length 6 cm.

FIG. 3. Peribronchial, esophageal, and subdiaphragmatic nodes, greatly swollen. Actual length 21 cm.

FIG. 4. Group of enlarged partially necrotic nodes in the hilum of the liver. Note the great enlargement of the node beside the cystic duct. Actual length 16 cm.



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Goodpasture and House

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PLATE 55

FIG. 5. Stomach with pancreas attached. Across the lesser curvature is a great chain of swollen lymph nodes. At the upper left is a cut section through a large one showing pale areas of necrosis. Actual length 16 cm.

FIG. 6. Enlarged nodes in the hilum of the lung. Actual length 16 cm.

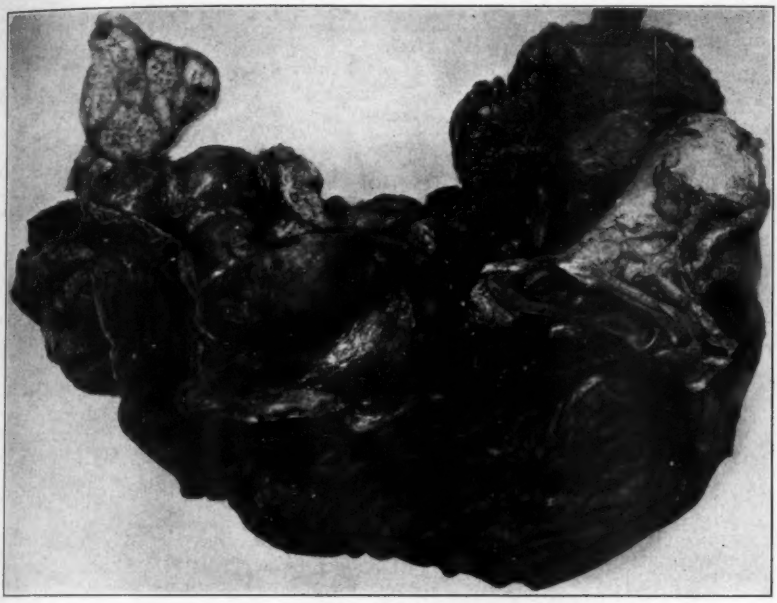

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PLATE 56

FIG. 7. Cut surface of the spleen showing the chalky opaque areas of necrosis. Actual length 16 cm.

7

PLATE 57

FIG. 8. Section through the base of the ulcer on the right index finger. It shows the depth of the necrosis and in places its perivascular arrangement. × 20.

FIG. 9. A magnification of the blood vessel at the lower right of Fig. 8, showing caseating necrosis and cellular reaction about a small artery. × 200.

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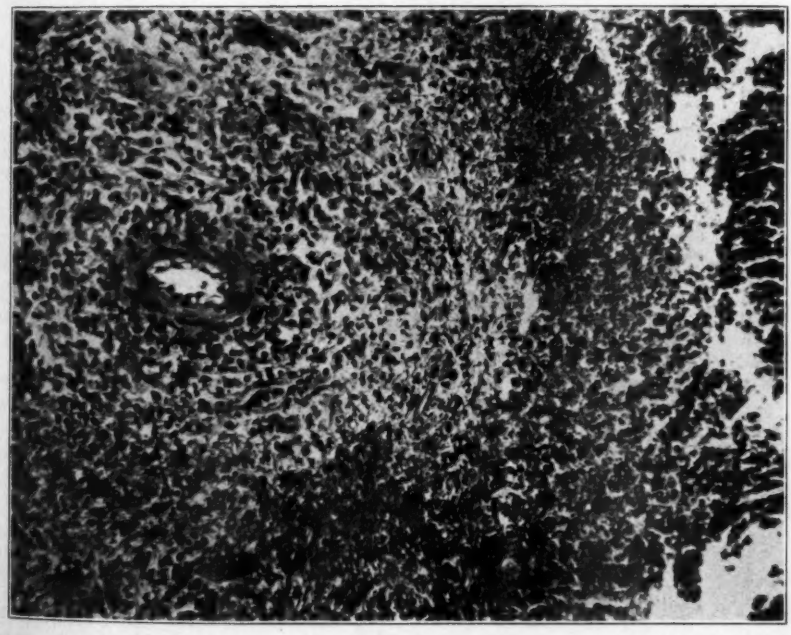

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PLATE 58

FIG. 10. Section through a small lymph node showing the extent and distribution of necrosis. ×20.

FIG. 11. A lymphatic sinus filled with cellular exudate. A cellular thrombus has formed in the right lower arm. This is the beginning of a necrotic focus. The cells are for the most part mononuclear phagocytes. × 200.

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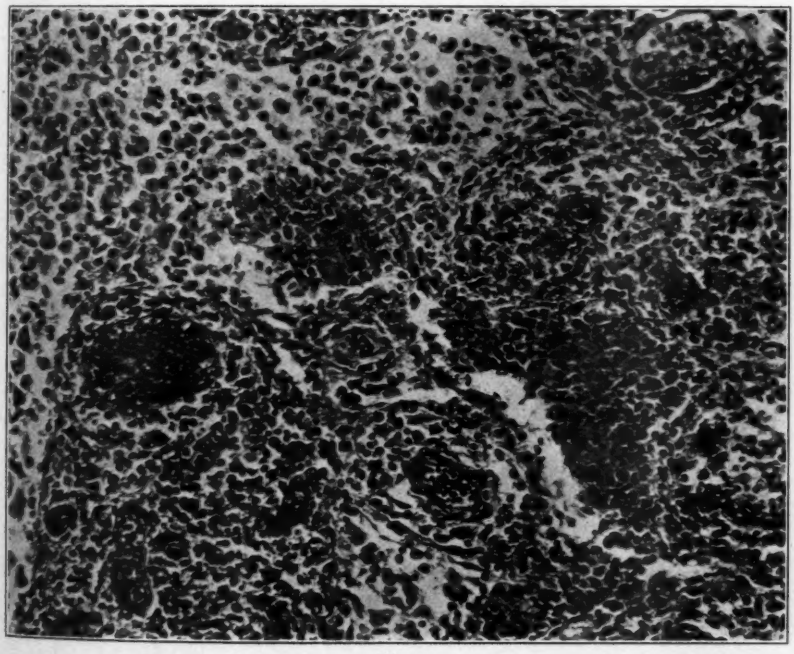

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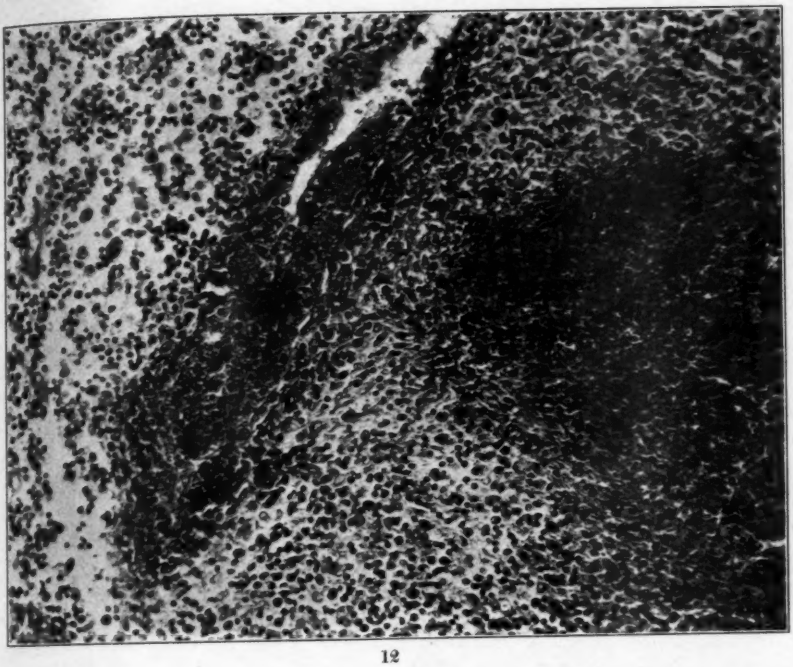
PLATE 59

FIG. 12. Thrombus in a blood vessel in the neighborhood of an acute necrotic focus in a lymph node. × 200.

FIG. 13. Necrotic focus with caseation in the liver. × 200.



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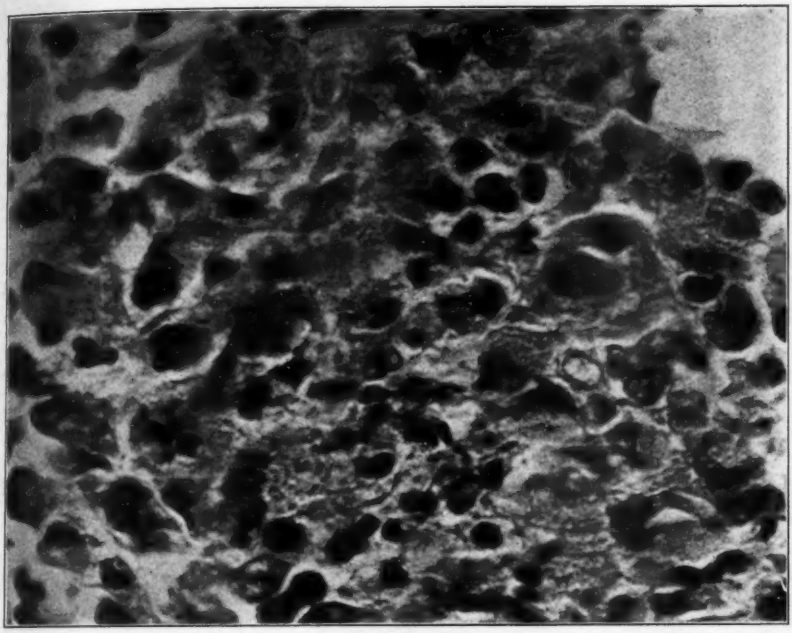
Goodpasture and House Pathologic Anatomy of Tularemia in Man

PLATE 60

FIG. 14. An early stage in the formation of an inflammatory focus in a lymph sinus. Almost all the cells are of the epithelioid type. Between them is a network of fibrin. × 1000.

FIG. 15. A beginning focus of necrosis in the parenchyma of a node. Many of the cells are polymorphonuclear leucocytes. Karyorrhexis of nuclei has begun. × 1000.



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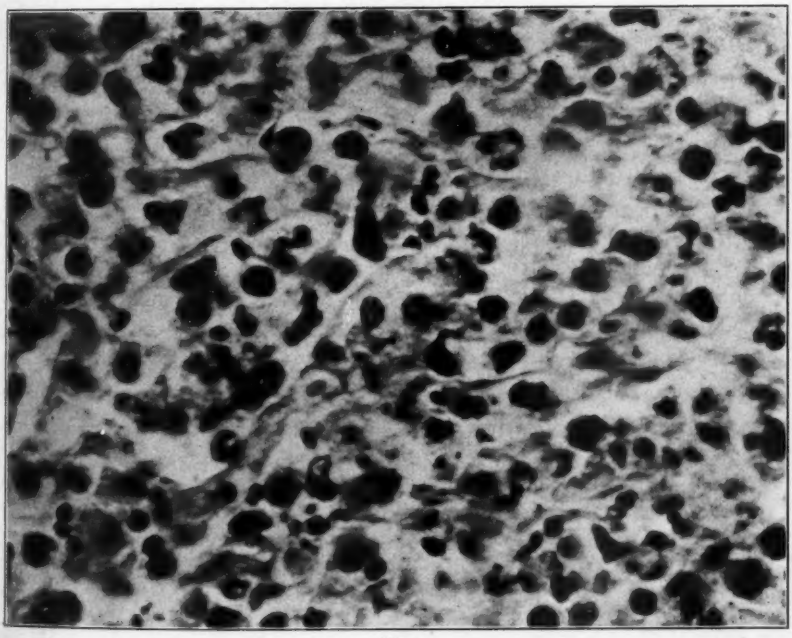

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Goodpasture and House Pathologic Anatomy of Tularemia in Man

STUDIES ON LIPOCHROMES *

I. THE REACTION OF ANIMALS TO THE PRESENCE OF CAROTIN

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The pigments commonly associated with the body fats and usually designated lipochromes have always been of considerable histologic and pathologic interest. However, plant physiologists and biochemists have been the most active in advancing knowledge concerning them, and little attempt has been made to apply the results of their work to human physiologic and pathologic problems. It is known that these pigments occur in relative abundance in certain organs normally, and that upon ingestion in unusual amount they impart a yellow color to the blood and skin. It is also known that in the course of certain metabolic disturbances (diabetes, malnutrition) they accumulate in certain organs to an unusual degree, but it has not been determined what rôle the lipochromes may play under such conditions, or whether they are more than casually associated with them. Most German writers (Oberndorfer [1]) believe that they constitute a type of "wear and tear" pigment associated with advancing age and are, therefore, autogenous (endogenous) in origin, but it has been shown by the biochemists that the lipochromes are probably always of exogenous origin and not a product of the metabolic activity of animal cells. The problem has not been subjected to extensive investigation from the pathologic point of view, and it was thought that by experimentation by both chemical and pathologic methods, the subject could be clarified somewhat.

The lipochromes which occur in animals consist chiefly of carotin and xanthophyll. That these are the same substances which occur in plants under similar names was shown by Willstätter and Escher [2] (1912) for carotin, and by Escher [3] (1913) for xanthophyll. They belong to a group designated carotinoids by Palmer.[4] He prefers the name chromolipoids, and defines them as pigmented substances having in general the solubility of the lipoids, occurring in cells in

* Received for publication March 16, 1928.

which lipoids are present, and like the phosphatides and sterols, absorbing oxygen readily. They are said to be stained by fat stains.

Carotin has been shown by Palmer and Eckles [5] to be the pigment present in cow-fat and milk. They identified the carotin of horse blood with the same pigment derived from plants, and Palmer and Kempster [6] showed that the pigment of egg yolk and of the skin of chickens (xanthophyll) came from their food and was identical with the xanthophyll of yellow corn. Van den Bergh and Snapper,[7] in 1913, showed that the yellow pigment of the human skin in "Xanthosis Diabetica" was associated with the presence of a similar pigment in the blood, and in later studies by Van den Bergh, Muller, and Broekmeyer,[8] the further fact that the pigment was the same as the carotin and xanthophyll of the ingested food was brought out.

Two attempts have been made to determine the reaction of the animal organism to these pigments when injected subcutaneously or otherwise. The first, by Wells and Hedenburg,[9] was to demonstrate a possible toxic action in guinea pigs. These animals were injected intraperitoneally with from 100 mg. to 200 mg. of carotin or chlorinated carotin in olive oil. This had no noticeable effect on the animals, and when killed four days later no lesions were found. The urine was described as of a deep reddish brown color. The intradermal injection of 20 mg. doses produced slight edema only. They concluded that "any such quantities (of carotin) as can ever accumulate in the tissues have no harmful effects." Hess and Meyers [10] injected carotin subcutaneously into infants. They described a yellow coloration of the plasma and urine following this, but no local lesion. The urine became yellower forty-five minutes after the administration of carotin in olive oil by mouth, and the color persisted for six hours. So far as I can find, these are the only reports of similar experiments.

Methods of Procedure

The technical difficulties encountered in the preparation of carotin in sufficient quantities for use in animal experiments are many, and after considerable preliminary experimentation the following modification of Escher's method was used.

Fifteen kilograms of carrots were sliced and dried at about 40° C in an oven. The dried carrots were powdered, extracted first with

alcohol, then by continuous extraction with petroleum ether or carbon disulphide. These solutions were evaporated, the first alcohol solution added, and the whole saponified with potassium hydroxide, and again evaporated. The residue was taken up in carbon disulphide, washed several times with 80 per cent alcohol, and spectroscopic examination made. This showed a wide band in the green-blue-violet which obscured the carotin bands; so the solution was evaporated and precipitated by absolute alcohol, the residue taken up in petroleum ether, and washed with 80 per cent alcohol until no further color could be removed. The petroleum ether solution then gave absorption bands at 495–475 uu. and 460–444 uu., corresponding with Willstätter and Stoll's [11] 492–476 and 459–445. The petroleum either solution was evaporated and the orange-red carotin taken up in 20 cc. of pure olive oil. The yield proved to be 26.8 mg. This yield is low because of rapid evaporation in a hood with the fan running, a process which must have oxidized a great deal of pigment which then became soluble in the alcohol. Some was lost in the absolute alcohol precipitation, and by discarding colored solutions which contained also considerable waxy substance, soap, and alkali. But a pure product was desired in order to determine standards for future work.

A more rapid method was used later to secure small amounts for immediate use. Carrots are sliced, boiled with 20 per cent potassium hydroxide in 70 per cent alcohol under a reflux condenser until they are mushy. The mixture is cooled and the alcohol poured off and discarded. (It contains practically no carotin.) The pulp is washed with 95 per cent alcohol in a Büchner funnel, with suction. The partially dried mass of carrots is mashed and mixed with anhydrous sodium sulphite or plaster of Paris, and this mixture is extracted with petroleum ether. A rapid extraction is now possible, limited only by the solubility of carotin in the solvent. Acetone or absolute alcohol may be added to the carrot-petroleum-ether mixture to take up any excess water, in the presence of which the carotin is not separated from the pulp completely. The petroleum ether solution is filtered off, the mass washed with petroleum ether, and the solvent then washed in a separatory funnel with 80 per cent alcohol until no pigment can be removed. The resultant petroleum ether solution responds to the chemical tests for carotin, and if washing has been thorough, the absorption bands in the spectroscope are not obscured

by impurities. Most of the carotin used in the following experiments was produced in this way.

Injection of Animals

A 500 gm. guinea pig was injected intraperitoneally with 2 cc., 3 cc. and 3 cc. of olive oil solution containing 0.67 mg. per cc. of carotin, receiving in all 5.36 mg. over a period of a month. The animal had no noticeable reaction. It was killed ten hours after the last injection. Between the time of the last injection and the killing of the animal two specimens of urine were secured, both only slightly yellow.

A small amount of bright yellow liquid oil remained in the peritoneal cavity, staining the peritoneum everywhere. Small masses of fatty material, yellow in color, contrasting sharply with the normal colorless fat of the mesentery, were present in the omentum and spleen. Most of these nodules were only lightly attached, but several upon the capsules of the liver and spleen were firmly adherent. A few small tubercles were present embedded deeply in the capsule of the liver, and similar, but smaller, pinpoint-sized nodules formed fine lines along the omentum and under surface of the diaphragm, apparently following the course of lymphatics. These lines could be easily seen owing to their yellow color. No nodules were present on the thoracic side of the diaphragm, or in or around the lungs. A mass of yellow fat was firmly attached to the lower (medial) pole of the spleen, but this did not extend for any distance into the substance of the organ, and no pigment was visible within the spleen. The normal retroperitoneal, mesenteric, and all other fat of the guinea pig was colorless as usual. The kidneys, aorta, and other organs excepting the adrenals showed no change, and these latter organs only enlargement. Side by side with the adrenals of a normal guinea pig, those of the carotin-injected animal appeared twice the size. This enlargement was undoubtedly due to the olive oil, rather than the carotin. A control guinea pig, receiving olive oil alone, had also visibly enlarged adrenal glands, with masses of partly encapsulated olive oil in the omentum and mesentery as the only other finding. This enlargement has been noted too often in fat and cholesterol feeding to be commented on here.

Blood, bile, urine and feces were examined from the carotin-injected guinea pig, as follows: Blood, 15 cc. plus 35 cc. 95 per cent

alcohol, was allowed to stand thirty minutes after shaking, until the precipitate had settled. The almost colorless supernatant fluid was poured off, and the precipitate treated with 60 cc. of petroleum ether. No color was extracted. The bile and urine, the latter only slightly yellow, were treated with alcohol, and extracted directly with petroleum ether. No color was extracted. Ten grams of feces from the rectum, colon, and cecum were extracted with petroleum ether after washing with alcohol, and a fair amount of yellow pigment obtained which could not be washed out with water or 80 per cent alcohol and gave the chemical tests for carotin. The animal had, however, been on a diet of hay and oats, with carrots until two weeks before its death.

Sections of spleen, kidney, adrenal and liver were examined unstained or stained with Scharlach R or Nile blue sulfate after fixation in formalin. The spleen section contained a granulomatous nodule attached to the capsule. This showed olive oil in large and small drops surrounded by endothelial cells and fibroblastic tissue. The oil stained red to orange with both stains in most places, but some drops were brownish red from the contained carotin. Many yellow crystals were present in the center of the lesion surrounded partly by neutral fat, and obscured by it when stained by Scharlach R. Considerable pigment was present in the spleen, and remained after thorough washing in acetone and chloroform.

The liver section showed also some adherent granulation tissue, but no lesion inside the capsule. The epithelium of the kidney tubules (collecting and Henle's tubules) contained numerous fine droplets which stained blue with Nile blue sulfate. The adrenal gland contained an immense amount of neutral fat and blue lipoid, but it could not be seen that the lipochrome content had increased.

The control guinea pig which received only olive oil intraperitoneally did not have any granulomatous lesions. This oil produces little or no reaction, remaining in the peritoneal cavity as a liquid for several weeks. Small masses of it may be covered by a thin membrane, forming a thin-walled fatty cyst; but it does not produce a granulomatous lesion. Hence, that type of reaction was attributed to the carotin. Its behavior, then, in this animal, was as a foreign body, slowly soluble and absorbable over a long period of time.

Two guinea pigs were injected intraperitoneally each with about 2 mg. of carotin (it is to be remembered that this amount of pigment

would color several liters of petroleum ether a distinct yellow), this time in aqueous suspension. Two hours later specimens of urine were secured. These contained no pigment recognizable as carotin. One of these animals (Guinea pig 2) was killed ten hours after injection. The urine, bile, and blood contained no carotin. Much of the pigment remained unabsorbed in the peritoneal cavity. The second animal (Guinea pig 3) was given a second injection of 10 mg. of carotin in aqueous emulsion with soap, and a third injection a day later of about 200 mg. Four hours and eighteen hours later 4 cc. of blood were taken from the heart. The urine was saved during this time, but no carotin could be extracted from it or from the blood.

Guinea pig 3 was killed three months after the last injection of carotin. There was an adhesion between a loop of intestine and the anterior abdominal wall, formed by scar tissue, in which yellow pigment could be seen. Several yellow nodules were present on the surface of liver and spleen, and on the omentum. The fat of the animal was colorless as usual. Sections of the nodules showed a fibroblastic phagocytic cell proliferation around crystals of pigment which were soluble in chloroform after dehydrating with acetone. They became decolorized when treated with ferric chloride solution for several hours. They did not stain with fat stains. As I have shown elsewhere, these are the only methods which can be depended upon to demonstrate carotin in tissues.

Two other guinea pigs injected with similar amounts of pure carotin showed no reaction to this substance other than that described above. It could not be shown that the carotin appeared in the blood or urine at any time following the injections. Wells and Hedenburg did not identify the brownish material in the urine of the animals which they injected. Hess and Meyers, however, evaporated urine of the injected infants to a small volume, extracted it with petroleum ether, and recovered a pigment which they assumed to be carotin. Carotin is not soluble in urine, and if present would probably be dissolved in a lipoid of some sort. It is hardly likely in that case that it could have been extracted directly from untreated, unsaponified urine with petroleum ether. But "urochrome" and bile pigment are also not soluble in petroleum ether. I secured brown urine from a guinea pig (Guinea pig 6) after the injection of 300 mg. of impure carotin. This was secured by the direct extraction of untreated carrot with petroleum ether, and evaporation of the solvent.

Carrots contain, in addition to carotin, xanthophyll and chlorophyll, and brownish and yellowish waxy substances, some of which are removed by saponification, but others only by careful washing and precipitation of the extract. The material injected into Guinea pig 6 contained all these substances, and one or several of them appeared in the urine when collected six hours later. The urine imparted a very faint color to petroleum ether, which, however, could be washed out with 90 per cent methyl alcohol. Its nature was not determined.

Two guinea pigs were fed each with 5 cc. of an olive oil solution of carotin, each receiving 3.35 mg. of carotin. Their urine was saved for the next twenty-four hours, but no carotin appeared in either specimen. Four hours after feeding, 5 cc. of blood were withdrawn from the heart of each animal, and examined for carotin. Both were negative.

A rabbit was injected intravenously on three successive days with small amounts of pure carotin in fine emulsion with soap. The exact amount could not be determined, but well-colored suspensions were used. These produced no noticeable reaction. The urine remained uncolored, and ten hours after the last injection there was no carotin demonstrable in the blood. It was killed ten days later, and no lesions found. The fat, which might possibly be expected to be stained by the pigment, was not noticeably changed in color.

Discussion

Carotin is soluble only in powerful lipoid solvents such as carbon disulphide, petroleum ether, ether, and chloroform and in lipoids themselves. It is an unsaturated hydrocarbon, $C_{40}H_{56}$, and is relatively inert chemically. It combines with the halogens and oxygen, and is broken down by strong mineral acids. It would seem that such a substance should behave in the animal body exactly as it has shown itself to behave in these experiments, that is, as a non-toxic, non-diffusible, slowly absorbable substance, forming a characteristic foreign body type of reaction in the tissues. These experiments are not strictly parallel with those of Hess and Meyers. Guinea pigs and rabbits do not normally contain carotin in the blood and none is present in the fat of these animals. It is possible that they may therefore react differently to the ingestion of carotin, though it does

not seem likely that their reaction to this pigment when injected into tissues would differ greatly from that of the human. These and other aspects of this problem will have to be discussed in later papers.

Summary

Carotin, the principal lipochrome found in the animal body, was extracted from carrots and purified. Upon injection intraperitoneally into guinea pigs granulomatous lesions were formed similar to those of familiar foreign body reactions. Carotin did not appear in the blood or urine of guinea pigs after injection or ingestion of relatively large amounts. It produced no effect in a rabbit when injected intravenously.

REFERENCES

1. Oberndorfer, S. Die pathologischen Pigmente. *Ergebn. d. allg. Pathol. u. path. Anat.*, 1921, xix, 47.
2. Willstätter, R., and Escher, H. H. Ueber das Lutein des Hühnereidotters. *Ztschr. f. physiol. Chem.*, 1911–12, lxxvi, 214.
3. Escher, H. H. Ueber den Farbstoff des Corpus luteum. *Ztschr. f. physiol. Chem.*, 1913, lxxxiii, 198.
4. Palmer, L. S. Carotinoids and related pigments. Chemical Catalogue Co., New York, 1922.
5. Palmer, L. S., and Eckles, C. H. Carotin — The principal natural yellow pigment of milk fat, etc. *J. Biol. Chem.*, 1914, xvii, 191.
6. Palmer, L. S., and Kempster, H. L. Relation of plant carotinoids to growth, fecundity, and reproduction of fowls. *J. Biol. Chem.*, 1919, xxxix, 299.
7. Van den Bergh, H., and Snapper, J. Die Farbstoffe des Blutserums. *Deutsches Arch. f. klin. Med.*, 1913, cx, 540.
8. Van den Bergh, H., Muller, P., and Broekmeyer, J. Das lipochrome pigment in Blutserum und Organen, Xanthosis, Hyperlipochromämie. *Biochem. Ztschr.*, 1920, cviii, 279.
9. Wells, H. G., and Hedenburg, O. F. The toxicity of carotin. *J. Biol. Chem.*, 1916, xxvii, 213.
10. Hess, A. F., and Myers, V. C. Carotinemia: a new clinical picture. *J. A. M. A.*, 1919, lxxiii, 1743.
11. Willstätter, R., and Stoll, A. Untersuchungen über Chlorophyll. Julius Springer, Berlin, 1913.

STUDIES ON LIPOCHROMES *

II. THE IDENTIFICATION OF CAROTIN, XANTHOPHYLL, AND ASSOCIATED LIPOIDS IN TISSUES

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Early in the course of a study of lipochromes it became evident that a review of the methods by which these substances are identified in tissues was necessary. The common impression among histologists is that these pigments are differentiated from other substances by their positive reaction with the usual fat stains. But it was soon found that they were frequently masked by the fat surrounding them or in which they were dissolved, and that, if they stained at all, they could not be differentiated from other lipoid substances which stain an identical color. Also, other crystalline pigments frequently adsorb some of the stains, so that they have the appearance, at least in part, of having been stained by these dyes. The following details were therefore investigated and are described for several reasons. First, a method for positively identifying lipochromes was desirable in order to make future work on these pigments possible. Second, a method for differentiating them from other lipoids in the body is necessary for the study of pigments in general. Third, since lipochrome is almost constantly associated with some other lipoid substance, it is desirable to know what that lipoid is, and whether it is always the same substance in all places where lipochromes are found. And finally it was thought necessary to clarify the subject of lipoid staining in general, as this has always been a puzzle to the writer, and seems to be to most pathologists.

Mallory and Wright [1] say that free carotin does not stain, but that the fat in which it is dissolved takes the usual fat stains. They give Smith's method with Nile blue sulfate, however, as staining the lipochrome blue. Smith [2] states that Nile blue sulfate differentiates fatty acids from neutral fats in the same manner that it appears to differentiate carotinoids from neutral fats, *i. e.*, fatty acids and carotinoid stain blue, and neutral fats red. Dolley and Guthrie [3] assert

* Received for publication March 16, 1928.

that the lipochromes in plants and animals stain with Sudan III and Scharlach R, even after bleaching. In a second paper,[4] these writers say that Nile blue sulfate when used as a progressive stain "particularizes the lipochrome first as a deep blue," and stains neutral fat a salmon-pink even in the presence of lipochrome. Palmer and Kempster [5] report that carotin in frozen sections of carrots and xanthophyll of chicken skin stain blue with Nile blue sulfate. These writers state that lard in which carotin has been dissolved, chicken fat colored with xanthophyll, milk fat (containing carotin) and egg yolk (xanthophyll) are stained salmon-pink by this stain, the pigment presumably not staining characteristically in these solvents, or being masked by the neutral fats present. Hueck [6] says lipochrome stains blue even after oxidation with hydrogen peroxide. Borst [7] named the pigment which he found in certain organs lipofuscin because it stained with Sudan III and Scharlach R, and was of the opinion that it must therefore contain some sort of fat substance. According to Staemmler [8] it is also said to be stained with neutral red, Nile blue sulfate, osmic acid, by the method of Fischler and Smith-Dietrich, at least in part, and is thereby differentiated from melanin. This pigment (lipofuscin) was recognized by Lubarsch [9] who classified it along with melanin as "wear and tear" pigment (*Abnutzungspigmente*). Staemmler, however, from results with the silver nitrate method of Schreiber and Schneider and the iron cyanide method of Unna concludes that most of those pigments which have been called lipofuscin (lipochrome) are melanin, or forms of melanin. It is obvious that the staining properties of these pigments have not been agreed upon.

Experiments in Staining

Frozen sections of carrots, unstained, mounted in glycerine, show a surprising amount of yellow pigment within the cells. Most of this is in the form of rectangular crystals, but some fibers resembling fragments of cells are colored yellow. A few cells contain small vacuoles but most appear to be empty, and practically all are devoid of nuclei. When treated with Scharlach R, alcohol-acetone solution, for thirty minutes and washed in 70 per cent alcohol a large number of red droplets appear in the cells, obscuring, or perhaps staining the pigment; but much yellow pigment remains unstained in the cells. Sections of carrots were treated as follows and

stained for from thirty minutes to twelve hours in alcohol-acetone solution of Scharlach R.

1. Sections of carrots which had been extracted with alcohol, then with ether. Considerable color remained. The tissue contained yellowish red pigment, but less in amount than untreated sections. This would not stain; no fat droplets were visible.

2. Sections washed in alcohol-ether, 1:1 solution, then stained with Scharlach R. Some pigment remained, which was not stained.

3. A section stained with Scharlach R in which the pigment was obscured by or stained red by the dye was washed in alcohol-ether solution. All the dye was removed, but most of the pigment, unstained, remained in the tissue.

4. Slices of carrot were placed in olive oil until the oil became well colored. Sections, unstained, showed a few fine yellow undissolved crystals, but most of the pigment had become mixed with the fat which filled most of the cells. The fat droplets had assumed a brownish color. When the section was stained with Scharlach R most of the drops stained red, but some were brownish red, showing that the crystals were surrounded by a thin film of olive oil.

Sections of carrots after treatment in the ways mentioned above were stained with Nile blue sulfate. The stained untreated sections showed nearly everything to be stained but the pigment. The blue color of the cell walls formed a good background for the yellow pigment. A few sections stained overnight showed blue halos around some of the carotin crystals. After treatment with alcohol and ether this color was removed, and the pigment remained unstained. The pigment dissolved in olive oil was masked by this stain, the oil staining salmon-pink, or a brownish pink where the pigment seemed most abundant.

Carrots after treatment by Ciaccio's [10] method (fixed in potassium dichromate, formalin, acetic acid solution 24 hours, treated with 3 per cent potassium dichromate for 3 days, then washed in running water 24 hours) showed no different staining reactions to Nile blue sulfate and Scharlach R. Most of the pigment had not been oxidized by this method, and was still present as yellow crystals. The chromation method of Smith and Mair,[11] somewhat similar to the Ciaccio method as to preliminary treatment, but which is followed by a modification of Weigert's hematoxylin method, was not expected to yield any results, as it is supposed to stain only unsatu-

rated fats and fatty acids. Their directions were therefore not followed specifically, but sections after treatment by Ciaccio's method, which should achieve the same result, were stained with Weigert's hematoxylin. It could not be seen that anything but cellulose was stained in the sections.

Frozen sections of adrenal glands and the corpus luteum of an ovary were treated in exactly the same ways as the carrot sections. Details of these procedures may, I think, be omitted. It was found that in the adrenal gland, unstained, the pigment could be seen most prominently in the reticular and inner fascicular zones, where, when stained with Nile blue sulfate, it was more closely associated with a blue-staining material. The outer zones contained the most neutral fat and the least pigment. The pigment of the corpus luteum is more diffuse. (In neither adrenal nor corpus luteum could the pigment be resolved as crystals because of its fine dispersion.) With Nile blue sulfate the pigment is obscured by neutral fat and blue lipoids. Scharlach R, since it stains all lipoids present, and so obscures the pigment, has no differentiating value. After treatment, with fat solvents, the Nile blue sulfate stain brings out the pigments more plainly, as it stains cells and nuclei, and, the fat having been removed, the pigment is more easily visualized.

Preliminary studies with neutral red and safranin showed that these stains had no particular differential qualities when applied to this pigment. Osmic acid was not tried, as it was thought that no differentiation from fats could be made even if this substance were oxidized. Wells [12] says carotin is usually but not constantly affected by osmic acid; Herxheimer [13] says it always is.

Hueck, and Dolley and Guthrie state that after oxidation with hydrogen peroxide or ferric chloride, carotin stains with Nile blue sulfate. Both these methods were tried. Frozen sections of carrots, adrenal gland and corpus luteum were treated with 3 per cent hydrogen peroxide in a covered dish, and with a strong solution of ferric chloride on slides. The yellow pigment was oxidized in the course of a few days in hydrogen peroxide, and in a few hours by ferric chloride. It was still visible in reduced light in the carrot sections as pale crystals, but could not be found in the adrenal or corpus luteum in unstained sections. When stained with Nile blue sulfate, the blue of the pigment, if it stained at all, could not be differentiated from cell fragments, cellulose, nuclear granules, etc.,

in the carrot section, and could not be differentiated from other blue-staining material in the adrenal and corpus luteum sections. This procedure also oxidizes some neutral fat which then stains blue also, making the picture more confused than ever. However, the fading of the crystals or the disappearance of amorphous granules when treated with ferric chloride is evidence of the nature of the pigment. Hematoidin and hemosiderin are not acted upon by this substance, and melanin, some of which may be oxidized by prolonged treatment with ferric chloride, first becomes black. This was checked in the adrenal section which contained medullary pigment. The melanin was plainly visible long after the lipochrome had disappeared.

The Molisch method [14] of demonstrating carotinoids in plants has never been applied to animal tissues, according to Palmer. This consists of placing the plant (leaves, petals, etc.) in a 20 per cent solution of potassium hydroxide in 40 per cent alcohol, allowing it to stand in the dark for varying periods of time, and observing the crystals which are then visible under the microscope. When animal tissues are treated by such a strong solution of alkali they dissolve before crystallization of the carotin can take place. A 10 per cent solution of potassium hydroxide in 70 per cent alcohol was therefore tried, but the cells of the section (adrenal gland) were pretty well digested in 5 minutes. A 10 per cent solution of potassium hydroxide in 70 per cent alcohol plus an equal amount of 4 per cent formaldehyde was found to dissolve cells slowly and to produce the best results, which, however, were not particularly good. A section of adrenal gland in which a dispersed yellow substance was visible was treated for forty minutes in this solution. After washing in water and mounting in glycerine the yellow material was found to be denser in some places, and absent in others, having apparently been released from its former association with fat. Drying the section before mounting brought out the crystalline character of the pigment. When a section of heart muscle was treated the same way the result was inconclusive, as much amorphous pigment remained scattered in the muscle fibers. While this method would serve to reveal lipochrome when it is the only pigment present, and to differentiate carotin from chlorophyll in plant tissues, it could not be used to separate pigments of two or more kinds in animal tissues.

Smith [2] gives the following staining reactions of various lipoids

with Nile blue sulfate: neutral fats, red; cholesterol and esters, pale red or light blue; phospholipins, pale blue; soaps and fatty acids, deep blue; carotin, deep blue; melanin, yellow-green (not stained). This is the dye now most often used to differentiate various lipoids in tissues, but from the preliminary observations recorded above it seemed that this wide range of differential colors could not be depended upon with certainty. If, however, this dye could be found actually to distinguish specific lipoids, one of our questions, namely, the kind of lipoid with which lipochrome is associated, might be answered by its use. Accordingly the following experiments were tried:

1. A rabbit was injected intraperitoneally with pure cholesterol in as heavy a suspension as could be made with water, repeated doses being given over a period of a month until about 2 gm. of cholesterol had been injected. Granulomatous lesions were present in the omentum, upon the spleen and liver, and in the mesentery when the animal was killed. Frozen sections of these lesions showed a typical foreign body reaction around crystals of cholesterol. When stained with Nile blue sulfate many of the giant cells present were seen to contain a deep blue liquid material. The crystals did not stain. Many smaller phagocytic cells contained blue or purple to purplish red droplets. These had a blue center, and a purple or reddish rim. Whether the cholesterol in the giant cells immediately surrounding the crystals had been changed before or at the time of liquifaction or whether it was simply dissolved in the cytoplasm of these cells could only be guessed at, but it stained deep blue, not pale red or light blue. As it changed color in the smaller cells at a distance from the crystals, it apparently had become changed either by esterification or by admixture with neutral fat. As the centers of these droplets were blue and the peripheries red, it seems likely that the latter occurred, particularly in view of the results of the second experiment.

2. Cholesterol was mixed with an excess of acetic anhydride and this boiled for two and a half hours under a reflux condenser. After adding water and cooling, the precipitate was taken up in petroleum ether, from which long needles precipitated upon standing. These were washed with water, then 95 per cent alcohol several times, and dried. The melting point was about 114° C (M. P. of cholesterol 147–148° C). The cholesteryl acetate was injected (about 200 mg.)

in aqueous emulsion into a guinea pig, intraperitoneally. Eleven days later when the animal was killed small nodules were present which contained crystals of the cholesteryl ester. Small giant cells around these crystals contained a deep blue material when stained with Nile blue sulfate and the edges of many of the crystals where they were apparently undergoing solution were blue. Smears from the peritoneum contained considerable liquid blue-retaining material. A few pink globules were present in and outside of cells. A section containing normal neutral fat, used as a control, stained pink.

3. Similar experiments were made with oleic acid, and boiled oleic acid plus cholesterol. The latter was possibly cholesteryl oleate mixed with cholesterol. Their staining reactions were the same as with cholesterol and cholesteryl acetate. Soap and lecithin, upon injection, also stain blue.

4. The behaviour of Nile blue sulfate in certain solutions was determined. It was found that it acts as an indicator, and is blue when neutral or acid, and purplish red to orange-red when alkaline. According to Smith it is a mixture of a strongly basic oxazine which unites with fatty acids to form blue soaps, and a weakly basic oxazone which dissolves in neutral fat, and is orange-red in color. That it does not behave as an indicator in animal tissues is shown by this reversal of color reactions. In vitro the dye behaves toward fatty acids and soaps as in the body. A soap solution even though ordinarily alkaline is colored blue, as well as fatty acids (oleic, stearic); but neutral fats are not colored red. Neutral olive oil dissolves the crystalline dye with great difficulty, turning blue. Two drops of 0.1 normal sodium hydroxide turn it reddish brown, and two drops of oleic acid turn it blue again. Pure tristearin stains blue in vitro. However, when in a thin film on a glass slide, the neutral fat mixtures with the dye assume a purple to red color. In the colorimeter both the fat and soap mixtures show two colors, namely, blue and red. The red is removed by adsorption with filter paper in watery solution and from soap and fatty acid mixtures.

There seem to be therefore both in vitro and in vivo, only two reactions to this dye, namely, a selective reaction of soaps, fatty acids, cholesterol and its esters, and lecithin, with the blue, and a similar physical or chemical affinity of neutral fats for the red. It seems likely that purples, reddish blue, and other slight changes in color represent only admixtures of the blue-staining substances with

neutral fat, and not specific lipoids. This dye would not therefore be of great use in differentiating the type of lipoid with which lipochromes are associated, beyond ruling out neutral fat.

It was determined by means of a Nicol's prism attached to the microscope that carotin in carrots and adrenal gland, and xanthophyll, the lipochrome of chicken skin, are not doubly refractive (anisotropic). In carrot sections some crystals are associated with anisotropic fat, as determined by their double refractiveness in stained sections, but numerous small droplets or irregular transparent masses are present which are doubly refractive and which are not associated with carotin crystals. Furthermore, when all the fat is dissolved out with ether, the carotin which remains (being less readily soluble) is not anisotropic. This doubly refractive lipoid of carrots with which the carotin is sometimes associated is presumably phytosterol.

It was more difficult to distinguish the carotin from the abundant anisotropic fat of adrenal gland. Lipoids cannot be dissolved out without extracting most of the pigment also. But what pigment remained in the zona fasicularis after such treatment was not doubly refractive. In chicken skin, where recognizable as xanthophyll, this pigment is not doubly refractive. In this organ the pigment occurs as crystals in the outermost layers of the corneum. Deeper in the tissue it is more widely dispersed, and is associated with fatty substances which stain purplish red with Nile blue sulfate. The corneum itself is impregnated with a lipoid which stains with Scharlach R, and becomes pink in Nile blue sulfate preparations. In the Malpighian layer, pigment could not be differentiated from blue-to-purple-staining fat. A thin luminous line of anisotropic substance is present where the cells of the Malpighian layer and the keratinized outer cells meet. This is the zone which stains purple with Nile blue sulfate. It probably contains a mixture of cholesterol and neutral fat. The anisotropism shown in this layer disappears on warming the section, a phenomenon supposed to characterize cholesterol and its esters.

Palmer and Kempster found xanthophyll to stain blue in the deeper layers of chicken skin. I could not be certain that the blue- or purple-staining material in the Malpighian layers was lipochrome. In the deeper layers, this substance is so finely dispersed that it could not be recognized by the methods described above. Only in

the outer scales of the corneum could it be resolved as a distinctive substance. And here it did not stain at all, and was not doubly refractive.

Summary and Conclusions

By the use of the usual fat stains on frozen sections of carrots, adrenal gland, corpus luteum, and chicken skin after treatment with fat solvents and other reagents, and on tissues removed from animals after injection of cholesterol, cholesterol esters, fatty acids, soap, and lecithin, and by the use of other reagents which might be expected to distinguish lipochromes from other substances in tissues, an effort was made to differentiate carotin, xanthophyll, cholesterol and other lipoids from one another. This has not been entirely successful, but the results justify the following conclusions:

1. Carotin and xanthophyll, the two common lipochromes, do not stain by any of the fat stains ordinarily employed to differentiate them.

2. When associated with a lipoid which does stain, this lipoid takes a blue stain with Nile blue sulfate, and may therefore be cholesterol, cholesterol ester, soap, fatty acid, or lecithin.

3. Because the substance with which the lipochrome is associated is usually anisotropic, and carotin and xanthophyll are not, this substance is assumed to be cholesterol or an ester of cholesterol.

4. When a pigment is present in tissues associated with a substance which takes a specific stain for fat, this pigment may be assumed to be a lipochrome, but this is not invariably so.

5. Yellow pigment which tends to coalesce when treated with weak alcoholic potash and formalin, or which crystallizes in the tissue after such treatment, is probably lipochrome.

6. Pigment which disappears or loses its color after treatment with a strong solution of ferric chloride (or other oxidizing agent), if not continued over too long a time, may be assumed to be lipochrome.

7. Pigment which is soluble in the usual fat solvents (ether, petroleum ether, chloroform, etc.) is probably lipochrome, but this treatment must be prolonged, and used after dehydration of the tissue with alcohol or acetone to be effective and of differential value.

8. Nile blue sulfate differentiates only neutral fats from all other lipoids, neutral fat staining red, and all other lipoids, which stain, blue. Lecithin, which takes a somewhat lighter stain than the other

blue-staining lipoids, may possibly be differentiated from them but careful timing of the staining process is necessary.

REFERENCES

1. Mallory, F. B., and Wright, J. H. Pathological Technique. Saunders, Philadelphia, ed. 8, 1924, pp. 211, 184.
2. Smith, J. L. On the simultaneous staining of neutral fat and fatty acid by oxazine dyes. *J. Path. & Bact.*, 1907, xii, 1.
3. Dolley, D. H., and Guthrie, F. V. The pigmentation of nerve cells, II — The lipochrome, a plant carotinoid pigment. *J. M. Res.*, 1919, xl, 295.
4. Dolley, D. H., and Guthrie, F. V. The pigmentation of heart muscle. *J. M. Res.*, 1921, xlii, 289.
5. Palmer, L. S., and Kempster, H. L. The physiological relation between fecundity and the natural yellow pigmentation of certain breeds of fowls. *J. Biol. Chem.*, 1919, xxxix, 313.
6. Hueck, W. Pigmentstudien. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1912, liv, 68.
7. Borst, cited by Hueck, Ref. 6.
8. Staemmler, M. Untersuchungen über autogene Pigmente. *Virchows Arch. f. path. Anat.*, 1924, ccliii, 459.
9. Lubarsch, O. Ueber feltthaltige Pigmente. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1902, xiii, 881.
10. Ciaccio. Method from Mallory and Wright, Ref. 1, p. 186.
11. Smith, J. L., and Mair, W. An investigation of the principles underlying Weigert's method of staining medullated nerve. *J. Path. & Bact.*, 1909, xiii, 14.
12. Wells, H. G. Chemical Pathology. Saunders, Philadelphia, ed. 5, 1925, p. 534.
13. Herxheimer, G. Abderholden's Handb. der biochem. Arbeitsmethod, 1913, vii, 686.
14. Molisch, H. Berlin botan. Ges. 1896, xiv, 18–29. Method from L. S. Palmer, Carotinoids and related pigments. The Chemical Catalogue Co., New York, 1922.

ANGIOMA RACEMOSUM VENOSUM — REPORT OF A CASE *

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The explanation for the sudden death of a young pregnant woman was found at necropsy to be an intraventricular hemorrhage from the rupture of a vessel of an angioma racemosum venosum — a rare abnormality of the vascular system of the brain.

The rareness of the lesion, the unusual circumstances and the opportunity of presenting a histologic study of the lesion prompted the following report.

REPORT OF CASE

Clinical History: The patient, a white female, aged 23 years, was in about the eighth month of her first pregnancy and apparently in excellent condition. During the two months preceding admission the patient had fallen five separate times while walking. Preceding each fall, the patient experienced a sudden loss of strength in the legs. The last fall occurred five days before admission. During the attacks there was no failure of vision, no pain, no dizziness and no loss of consciousness. Strength returned in about five minutes, and the patient was always able to resume the walk. Nothing in the past history appeared to be related to the present condition.

On the morning of admission the patient felt in her usual good health. Shortly after arising and while at the toilet the patient suddenly cried out that she was blind and then fell unconscious. Two hours later the patient was brought to the New Haven Hospital and died there shortly after admission. The patient was unconscious when admitted. The pulse rate was 76 per minute; the blood pressure, systolic 100, diastolic 60; the temperature 99° F; and the respirations were stertorous and slow (8 per minute). The body was cold and cyanosis was extreme. There was no subcutaneous edema. Numerous moist rales were heard in the lungs. There were no convulsions. The urine examination showed a very faint trace of albumen and a few hyaline casts.

Necropsy Report: The post-mortem examination was made three hours after death. The findings explaining the cause of death are confined to the brain.

The blood vessels of the superior and lateral surfaces of the brain are distended with blood and the subarachnoid space contains enough blood-tinged fluid to give the surfaces a bright pink color. The basal cisterns are distended with blood, which was aspirated

* Received for publication February 16, 1928.

from the ventricles before the brain was opened. After injection with and fixation in 10 per cent formalin the sections of the brain show the ventricles to be filled with clotted blood. A single, non-encapsulated, vascular, spongy tumor occupies the greater part of the right occipital lobe (Fig. 1). The accompanying pictures show the exact location. The tumor is made up of thin-walled vessels of various caliber surrounded by a translucent gray-white tissue. The largest vessel is 7 mm. in diameter and is located in the posterior medial portion of the tumor. This vessel is connected with a number of the smaller vessels of the tumor and also with two thin-walled pial vessels. The latter vessels are approximately 2 mm. in diameter. The pial vessels adjacent to the tumor are larger than the pial vessels of the opposite occipital lobe. The connection of the smaller vessels of the tumor and of the adjacent pial vessels to the large vessel is demonstrated by injecting a gelatin mass into the large vessel and watching the smaller vessels fill. With the aid of a lens, focal thickenings of the vascular walls are seen. No thrombosed vessels are found. Several small calcareous deposits are felt in the vessel walls. In the posterior horn of the right lateral ventricle the vessels of the tumor have pushed up the ependymal lining and one vessel is seen with a tear about 1 mm. in length which is coated over with recently clotted blood.

Microscopic Examination: Sections from the lesion were stained with Mallory's phosphotungstic acid hematoxylin, hematoxylin-eosin, Weigert and Verhoeff's elastic tissue methods and Perdrau's connective tissue method.

The lesion is composed of a large number of blood vessels of varying size and shape. The largest vessel (Fig. 2) measures 11 mm. in its greatest diameter while many other vessels are from 3 to 7 mm. in diameter. The vessel walls vary greatly in thickness. The greater portion of all of the vessels resemble medium-sized or small veins. There are, however, numerous fusiform or nodular thickenings of the intima occasionally, but most often of the media, which, in Fig. 2, are seen to occur gradually or sharply at irregular intervals along the otherwise thin-walled vein. The phosphotungstic acid hematoxylin stains bring out the fact that mygolia fibrils (Fig. 3) predominate in the thickenings of the media although collagen, fibroglia and elastic fibrils are also seen.

The intima is composed of a single layer of endothelial cells.

Scattered, irregular subintimal thickenings are composed of loosely arranged cells resembling young connective tissue cells (Fig. 5). An incomplete internal elastic layer is found in the thickened portions of several large vessels (Fig. 4), although small fragments of elastic tissue are seen in other vessels. The media of several of the large veins contains small calcium deposits. The local nodular and the fusiform thickening of the media, projecting into the lumen, are described above. The adventitia is thin. No cells of mesodermal origin are found between the vessels of the lesion. The greater portion of the tissue between and at the periphery of the vessels is composed of dense neuroglia fibrils and degenerated brain tissue.

No evidence of hematopoiesis is seen in the sections but smears from the blood of the tumor were not made.

Discussion

The lesion described is considered to be a congenital anomaly of the vascular system although it is named angioma racemosum venosum. Because of the variations in nomenclature and the absence of verification by histologic methods, difficulty has been experienced in selecting cases from the literature. There are but three recorded instances of angioma racemosum venosum which were verified by histologic study.* Thirteen reported cases seen at operation and one at necropsy presented the typical gross appearance of this type of vascular abnormality — a collection of non-pulsating, tortuous, thin-walled vessels distended with dark blue blood — but no microscopic examination was made. Another example of the rarity of the lesion is the fact that among 1500 verified cases of intracranial tumors Dr. Harvey Cushing has seen at operation only five examples of angioma racemosum venosum and in none of these cases was it possible to remove tissue for histologic study.

The lesion has not been diagnosed clinically. It has been usually seen at exploratory cranial operations and recognized by the previously mentioned characteristics. In the several cases examined at necropsy there has been noted a wedge-shaped collection of veins of various caliber which projected into the cerebral tissue for a varying distance from the superficial collection of veins. The histologic studies in the three reported cases showed changes in the vessel walls similar to those described in this report.

* See Bibliography: Dürck, E. Herzog, E. Therman.

No previous report has been found of rupture of a vein of an angioma racemosum venosum into the brain or ventricle with subsequent hemorrhage and death.

BIBLIOGRAPHY

Beadles, C. F. A telangiectasis of the left frontal lobe, with epileptiform convulsions. *Arch. Neurol. & Psychiat.*, 1899, i, 440.

Campbell, H., and Ballance, C. A. A case of venous angioma of the cerebral cortex. *Lancet*, 1922, i, 10.

Castex, M. R., and Bolo, P. O. Angioma venosum racemosum der linken motorischen region. *Deutsche Ztschr. f. Nervenh.*, 1914, lii, 356.

Christiansen, V. Les tumeurs du Cerveau. Paris, Masson et Cie, Ed., 1920, case p. 66.

Dürck. Ueber ein grosses plexiformes venöses angiom der weichen Hirnhäute mit uebergreifen auf den linken grosshirnscheitellappen. *München. med. Wchnschr.*, 1907, liv, 1154.

Hammes, E. M. Intracranial telangiectases. *Arch. Neurol. & Psychiat.*, 1921, vi, 263.

Herzog, E. Angioma racemosum venosum des Schädels und gehirns. *Beitr. z. path. anat. u. z. allg. Pathol.*, 1927, lxxvii, 312.

Krause, F. Krankenvorstellungen aus der Hirnchirurgie. *Zentralbl. f. Chir.*, 1908, xxxv, 61.

Magnus, V. Bidrag til Hjernechirurgiens Klinik og Resultater. Kristiania, 1921, Trykt. i Merkur, p. 101.

Morris, H. Venous vascular tumor of cerebrum. *Tr. Path. Soc.*, London, 1871, xxii, 22.

Orbison, T. J. Angioma racemosum of the pia, with epileptoid convulsions of the Jacksonian type. *J. A. M. A.*, 1915, lxiv, 1575.

Rotgans, J., Hers, et Winkler, C. Angiome de la pie-mère situé sur la zone motrice, Ablation partielle, Amélioration. Chipault: L'Etat Actuel de la Chirurgie Nerveuse, Paris, 1902, i, 694.

Sachs, E. Intracranial telangiectases: symptomatology and treatment, with report of two cases. *Am. J. M. Sc.*, 1915, cl, 565.

Therman, E. Ein fall von angioma racemosum cerebri und ein fall von pachymeningitis mit obliteration sinum durae matris. Arbeiten aus der pathologischen Institut der Universität Helsingfors, 1910–1913, iii, 67.

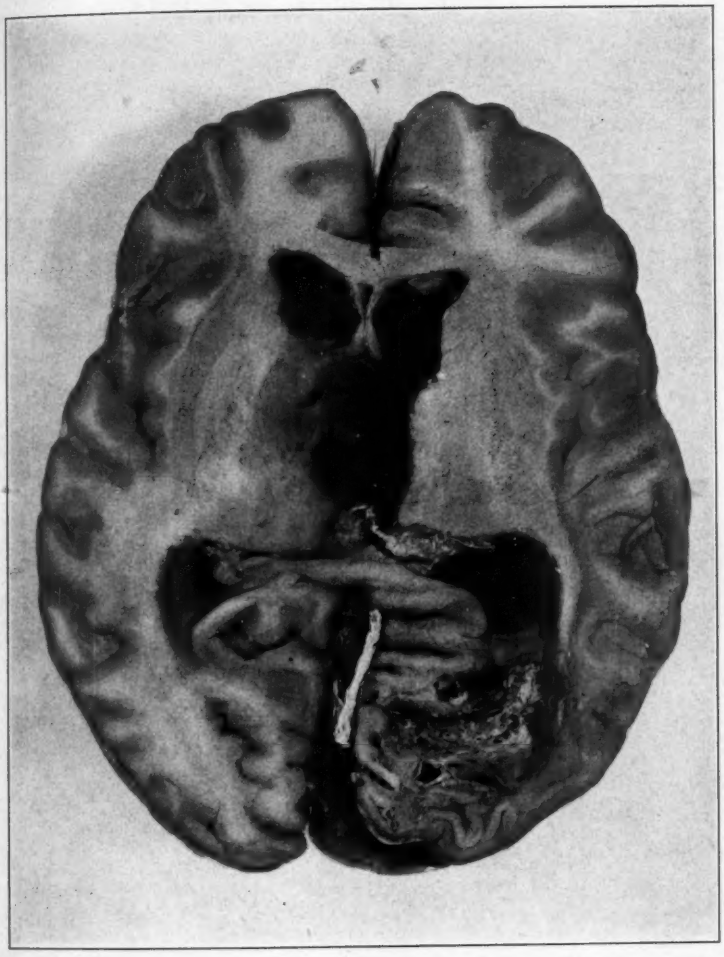
Worster-Drought, C., and Ballance, C. A. Venous angioma of the cerebral cortex, with report of a case. *Lancet*, 1922, ii, 125.

Worster-Drought, C., and Carnegie-Dickson, W. E. Venous angioma of the cerebrum. Report of a case with necropsy. *J. Neurol. and Psychopath.*, 1927, viii, 19.

DESCRIPTION OF PLATES

PLATE 61

FIG. 1. Abnormal collection of vessels in the right occipital lobe with rupture and hemorrhage into the lateral ventricles.



1

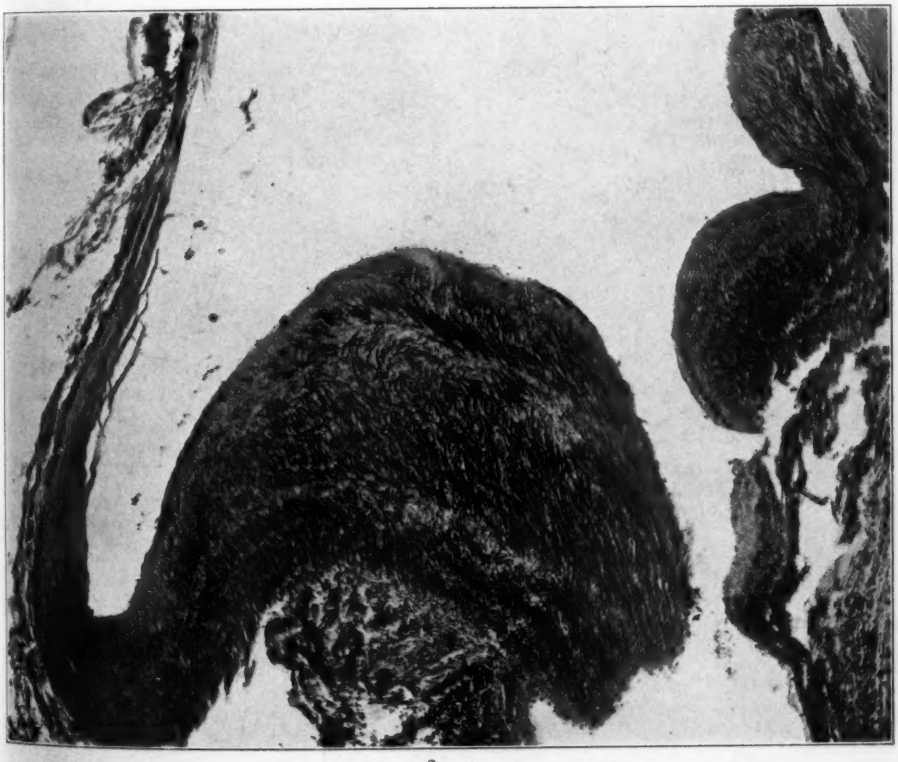
Buckley Angioma Racemosum Vènosum

PLATE 62

FIG. 2. Cross-section of large, irregular vessel seen in posterior medial portion of Fig. 1. Note the numerous subintimal and medial thickenings. H. and E. × 10.

FIG. 3. Leiomyomatous thickening of the media stained with phosphotungstic acid hematoxylin to show the myoglia fibers. × 80.

2



3

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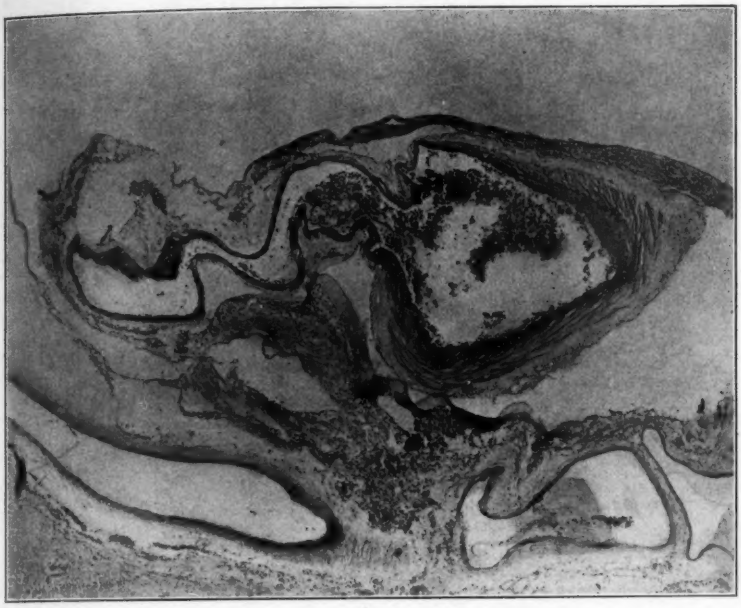
PLATE 63

FIG. 4. Irregularly shaped vessel stained by Weigert's elastic tissue method to show the incomplete internal elastic lamellae. × 20.

FIG. 5. Nodular subintimal thickening surrounded by degenerated brain tissue. Weigert's elastic tissue stain. × 30.



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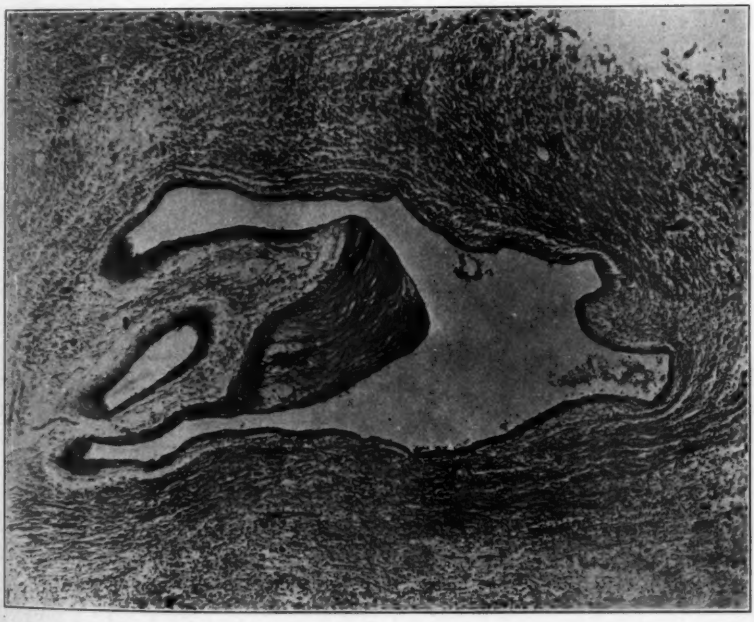

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Buckley

Angioma Racemosum Venosum

SPONTANEOUS RUPTURE OF THE HEART *

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Attention has been called to this subject by the recent review of the literature by Krumbhaar and Crowell,[1] and de la Chappelle.[2] The incompleteness of the publications reporting incidences of spontaneous rupture of the heart is emphasized. In the pathology service of the New Haven Hospital there have been three cases of spontaneous rupture of the heart which seem to be of sufficient importance to present in some detail.

CASE I. *Clinical History.* The patient, a white male, aged 35 years, came to the New Haven Dispensary on July 14, 1924, complaining of lameness, pain, numbness, coldness and prickling sensations occurring in the left lower leg and foot following a short walk. Walking could be resumed after a rest but the symptoms soon returned. The patient had had a drawing sensation over the precordium during the previous year, but otherwise was in excellent condition. The examination showed that the arteries of both legs and feet pulsated. There was, however, a faint erythema of the skin of the left big toe which was not present in the skin of the rest of the foot. The urine examination showed a trace of sugar which disappeared with dietary regulations. Rest was prescribed but there was no relief and the patient came into the hospital for observation. A periarterial sympathectomy was advised but refused and the patient was discharged. During the next month the original symptoms became more severe, and the patient reëntered the hospital. A periarterial sympathectomy of the left leg was successfully done. The patient's condition improved so that he was discharged on October 1, 1924. During the next three weeks the previous condition became worse and on return to the dispensary the patient was advised to reënter the hospital to have his big toe amputated. At this time the big toe of the left foot was dry and black. There was a line of demarcation with a reddened edematous skin on the dorsum. The other toes appeared normal. The dorsalis pedis and posterior tibial arteries could not be felt to pulsate. Five days after this admission an amputation of the left big toe was made. In the post-operative course the wound became gangrenous and did not heal. It was then decided to amputate at the middle of the thigh. On December 25, 1924, the patient was transferred to the medical service for treatment of the diabetes. It was found that on ten units of iletin per day the urine was sugar free. At this time the patient complained of pain in the left upper chest and of shortness of breath. A pericardial friction rub was heard which was present for a day and a half. Following this the patient had a temperature ranging

* Received for publication February 16, 1928.

from 100° to 102° F. The patient complained of considerable pain at the stump of the left thigh, and an excision of the sciatic nerve was made. Histologic examination of the nerve and surrounding tissue was negative. Following this operation symptomatic relief was obtained. The patient had considerable pain in his left shoulder, especially following pressure in the region of the apex of the heart. This pain was persistent in the left supraclavicular portion but also radiated to the upper portion of the left arm. The patient's condition was apparently improving when suddenly, on March 9, while sitting up, he fell forward and began to gasp. He was very cyanotic, the pulse was not felt, and the heart sounds were irregular with a rate of 40 per minute and a large auriculoventricular interval. The patient died shortly afterward. A clinical diagnosis of coronary occlusion as a terminal event was made.

Necropsy Report: The chief findings of the post-mortem examination are in the cardiovascular system and these only will be described. The pericardial sac is distended by about 600 gm. of fluid and clotted blood. The clotted blood is easily pulled off every part of the visceral pericardium except over the posterior part of the apical saccular dilatation. Here the pericardial adhesions are firm and the clot is lightly adherent to the junction of the adhesions and myocardium and to a small three-cornered rupture of the aneurysmal dilatation. The openings of the coronary arteries are patent. A dissection of the main branches of the right and left coronary arteries shows that before they divide their wall is raised here and there with small light yellow plaques which encroach only to a small extent upon the lumina. As the anterior descending branches of both arteries are dissected the luminae become smaller. A cross-section of the anterior descending branch of the left coronary artery 2 cm. from the beginning at this area shows an almost completely obliterated lumen with only a small, pin-point drop of blood exuding from the central portion of the artery. As the artery is followed down it is seen that this same condition holds to the apex. The descending branch of the right coronary artery shows a similar picture but even more extensive in that there is no lumen to be seen in any portion shortly after its origin. At the apex there is a sudden transition from the thick muscle wall to a thin fibrous tissue wall from 1 to 2 mm. in thickness which bulges out to form an oval-shaped dilatation measuring about 6 cm. in any diameter. This large aneurysmal dilatation is completely filled and distended with blood clots of varying ages. The center of this mass is made up of gray-white, very friable, clotted material which extends only a short distance into the cavity of the left ventricle. The parietal pericardium is firmly adherent to

the apex wall. On cross-section it is difficult to distinguish between the adherent pericardium and the wall of this dilated portion of the ventricle itself. Directly above this dilated area the endocardium of the left ventricle is pearl-white in color. The myocardium of the lower and middle part of the left ventricle is streaked with or almost completely composed of gray-white, dense, fibrous tissue. The wall of the right ventricle averages 6 to 7 mm. in thickness. The left ventricle wall measures 18 to 19 mm. in its upper half and 12 to 13 mm. in its lower portion just before it sharply changes to the wall of the aneurysmal dilatation. The sinuses of Valsalva are normal. The aorta, renal, and splenic arteries show considerable sclerotic change.

Microscopic Examination: Histologic studies are made of the myocardium, wall of the aneurysmal sac, and of the coronary vessels. There is complete occlusion of the coronary vessels supplying the lower half of the left ventricle, and infarction, mural thrombus formation, and a healing process by which a new wall at the apex is formed. Compensatory hypertrophy of the remaining cardiac fibers is present. The myocardium of the left ventricle is badly damaged. Dense masses of fibrous tissue and an increase of interstitial tissue represent the healed process. Necrotic cardiac fibers with opaque cytoplasm and pyknotic or absent nuclei represent the more recent process. Adherent organized or organizing thrombi with fresh thrombotic deposits are seen at the apex. The undamaged cardiac fibers are hypertrophied and their nuclei are peculiar in their increase in size and irregularity. Scattered through the myocardium and in the loose subepicardial tissue, there are large deposits of mononuclear and plasma cells about the vessel sheaths. The wall of the saccular dilatation is less than 1 mm. in thickness in places. It is made up of a very loose connective tissue which is infiltrated with mononuclear cells. Near the point of rupture there are extravasations of red blood cells into the recently organized wall. The descending branches of both coronary vessels are completely occluded by a dense, acellular, light pink tissue, the center of which contains several thin-walled capillaries. In the media of the left branch there are large calcium deposits.

Anatomic Diagnoses: Primary. Generalized arteriosclerosis with special involvement of: (*a*) posterior tibial and dorsalis pedis arteries; old operations (amputation of toe and left leg for gangrene; periar-

terial sympathectomy; resection of sciatic nerve, left); (*b*) coronary arteries with occlusion of descending branches, right and left; healed infarct of left ventricle; aneurysmal dilatation of infarcted area of left ventricle and rupture of its wall; hemopericardium; hypertrophy and dilatation of the left side of the heart; congestion of the viscera.

CASE II. *Clinical History.* A white man, about 60 years of age, collapsed on a street car and died en route to the hospital. No clinical history was obtained.

Necropsy Report: The post-mortem examination is made shortly after death. The principal findings are in the cardiovascular system. The pericardial sac is distended with about 100 cc. of fluid blood. The visceral pericardium is covered by an elastic, non-adherent, recent blood clot about 1 cm. in thickness except over an area 3 cm. in diameter on the anterior surface of the left ventricle where the pericardial layers are bound by easily separated fibrinous adhesions. The heart is enlarged and weighs 650 gm. There is a ragged tear 5 mm. in length on the anterior surface of the middle of the left ventricle 1 cm. to the left of the septum. This extends into the left ventricle and is filled with a friable clot which is continuous with a thrombus adherent to the inner ventricle wall about the ruptured myocardium. The subepicardial fat is thick in all areas. The myocardium about the point of rupture and at the apex of the left ventricle is thin, averaging 4 mm., and contains considerable gray-white scar tissue. Elsewhere the myocardium of the left ventricle averages 14 mm. in thickness. The anterior descending branch of the left coronary artery is almost entirely occluded 4 cm. from its origin except for a small pin-point lumen which is filled with a bright red clot.

Microscopic Examination: Histologic sections about the point of rupture show a fibrinous exudate over the pericardium, a recent mural thrombus attached to the endocardium, and necrosis of the cardiac fibers with an extravasation of red blood cells and an infiltration of polymorphonuclear leucocytes between the necrotic fibers. The margin of the rupture is coated with a fibrin network and red blood cells. There are other areas of fibrosis with hypertrophy of the remaining fibers at the apex. The coronary vessels show varying degrees of occlusion from the only slightly raised intimal thickening

to almost complete occlusion. The anterior descending branch of the left coronary artery has only a small slit left as a lumen. The intima of this vessel is thickened by light pink-staining, acellular tissue and an amorphous material containing many crystal spaces as well as calcium deposits. The media and adventitia are unchanged.

Anatomic Diagnoses: Primary. Generalized arteriosclerosis involving particularly the coronary arteries; cardiac hypertrophy and dilatation; chronic passive congestion of the viscera; fibrosis of the myocardium; infected infarct, thrombus, and rupture of the left ventricular wall; hemopericardium.

CASE III. *Clinical History.* A white female, aged 66 years, with a known history of diabetes mellitus was admitted to the New Haven Hospital on February 24, 1926, with a complaint of precordial pain of two weeks duration. Four days before admission the previously dull pain suddenly became severe enough to cause the patient to faint and hospitalization was advised. On admission the pulse was 100 per minute, irregular and of poor volume; the respirations were 20 per minute and the blood pressure, systolic 132, diastolic 82. There was no dyspnea or cyanosis. The cardiac boundaries were not made out. A systolic murmur was heard in the apical region. The important laboratory findings were a leucocytosis of 24,500 per c. mm. with 85 per cent polymorphonuclear leucocytes. The electrocardiogram showed a regular rhythm with an average rate of 110 per minute and an inversion of a T wave in lead 1. On the day after admission a house officer found the patient's general condition improved. Twenty minutes later the patient died suddenly. The clinical diagnosis was diabetes mellitus and arteriosclerosis with special involvement of the coronary arteries; occlusion of the coronary arteries; infarction and rupture of the heart with hemopericardium.

Necropsy Report: The pericardial sac is enlarged and distended with about 200 cc. of fluid blood. The visceral pericardium is coated with a layer of recently clotted blood which varies in depth from 1 to 5 mm. The clot is lightly adherent to an area 5 cm. in diameter over the middle of the anterior surface of the heart. This part of the myocardium is softer than the surrounding muscle and is coated over with a fibrin deposit. There is a small irregular break in the pericardial tissue, measuring 2 to 3 mm. in any diameter, which extends into the softened infarcted area. The rupture is covered and filled with a lightly adherent blood clot. Before the heart is opened the coronary arteries are injected with a barium chlorid-gelatin mixture, and stereoscopic X-ray pictures are made of the injected heart. The injection material leaks out through the rupture in the anterior descending branch of the left coronary artery. The injec-

tion of the right coronary artery shows no occlusion. The apex of the left ventricle shows very few injected vessels. The infarcted area is soft, opaque, brown-yellow, and is infiltrated with fluid and clotted blood.

Microscopic Examination: Just above the infarct the anterior descending branch of the left coronary artery is completely occluded by a recent thrombus and a thickened intimal and medial coat. Collections of mononuclear cells form dense collars about the vasa vasorum of the adventitia. The sections through the infarct show necrotic cardiac muscle fibers with vacuoles, loss of striation and nuclei, a diffuse and dense infiltration of polymorphonuclear leucocytes, acute inflammation of the vessel walls, a recent infected mural thrombus and an extravasation of red blood cells splitting up the cardiac fibers and extending out through the wall. A recently formed thrombus partly occludes the ruptured point. Sections elsewhere in the myocardium show an acute diffuse inflammatory process as well as areas of fibrosis and hypertrophy of the fibers.

Anatomic Diagnoses: Primary. Generalized arteriosclerosis involving especially the coronary arteries; fibrosis of the myocardium; thrombo-arteritis of the anterior descending branch of the left coronary artery; mural cardiac thrombus; infected infarct of the myocardium with hemorrhage and rupture; hemopericardium.

Discussion

Krumbhaar and Crowell's review of 632 cases reported in the literature up to 1925 shows that 72 per cent fell in the age group of 50 years upward and about 4 per cent in the 30 to 40 year period. The greater number of cases were associated with preceding coronary artery lesions with a resulting infarction and rupture of the infarcted area. Invariably the site of rupture was in the left ventricle and was associated with changes in the anterior descending branch of the left coronary artery. Krumbhaar collected seven cases in 16,000 necropsies at the Philadelphia General Hospital and quoted seven in 13,000 at Munich, and nine in 8,000 at Leipzig. De la Chappelle reported twenty such cases among 15,059 necropsies from the Bellevue Hospital (New York City) and Medical Examiner's records. The three cases reported here are the total from 1,330 autopsies. Among these there have been 150 cases in which diseases of the

coronary arteries and the myocardium were the primary causes of death. In all but three of these cases the anterior descending branch of the left coronary artery was especially involved. Aneurysms of the left ventricle are rarely found. Kahn [3] and Elliott [4] present a review of the cases up to 1922 and their description of the typical aneurysm is similar to that of Case I.

The cases reported here present several interesting features. Cases I and III have associated diabetes mellitus and evidence of infection of the cardiovascular system. The etiology of the infection is not clear in either case. The anterior descending branch of the left coronary artery is completely occluded in each case, and in Case I the descending branch of the right coronary artery is also occluded. Evidence of old and recent infection of these vessels is presented. Case I is of further interest in that the patient was only 35 years old.

REFERENCES

1. Krumbhaar, E. B., and Crowell, C. Spontaneous rupture of the heart. A clinico-pathologic study based on 22 unpublished cases and 632 from the literature. *Am. J. M. Sc.*, 1925, clxx, 828.
2. de la Chappelle, C. E. Spontaneous rupture of the heart; an analysis of fourteen cases. *Am. Heart J.*, 1925–26, i, 315.
3. Kahn, M. H. Aneurysm of the left ventricle. *Am. J. M. Sc.*, 1922, clxiii, 839.
4. Elliott, A. R. Cardiac aneurysm. *Med. Clinics of North America*, 1924, viii, 495.

DESCRIPTION OF PLATES

PLATE 64

FIG. 1. Occlusion of descending branches of the right and left coronary arteries; infarct of left ventricle; fibrosis of myocardium, rupture of aneurysmal dilatation of infarcted area; organizing and acute pericarditis.

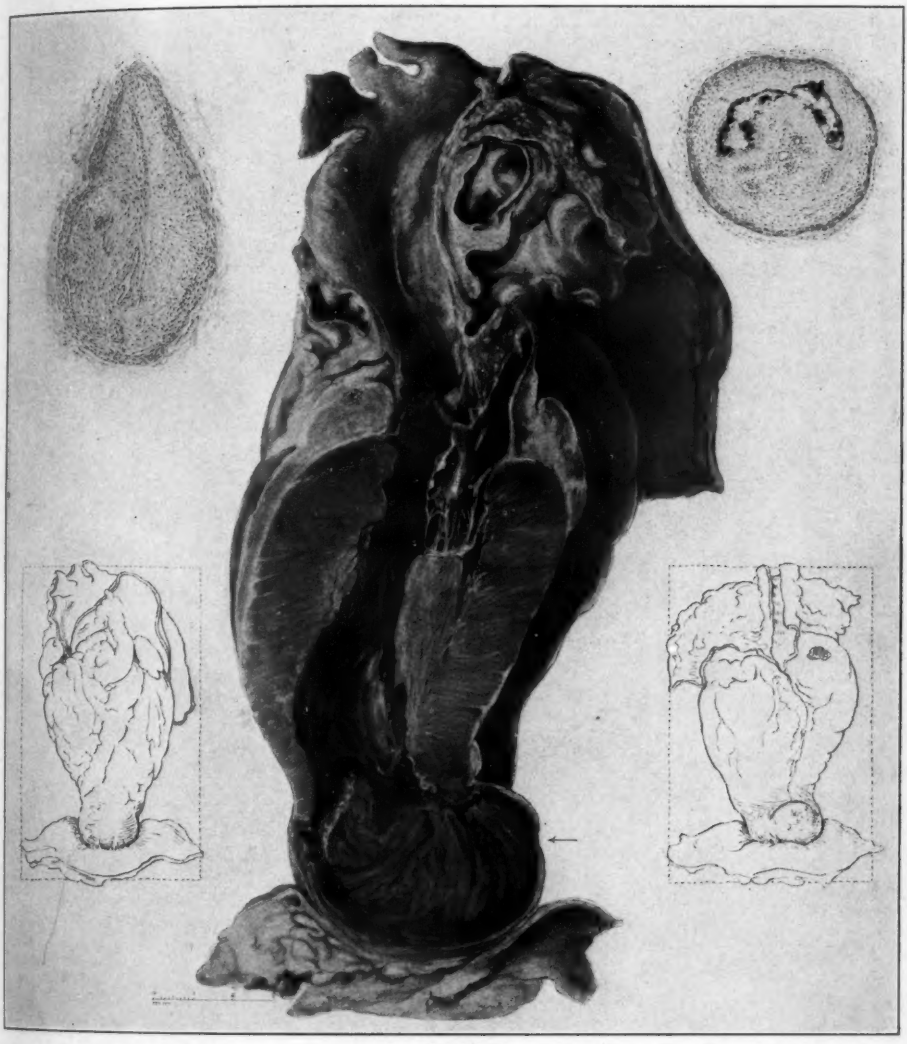

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PLATE 65

FIG. 2. Infected infarct, thrombus and rupture of left ventricle wall (A).

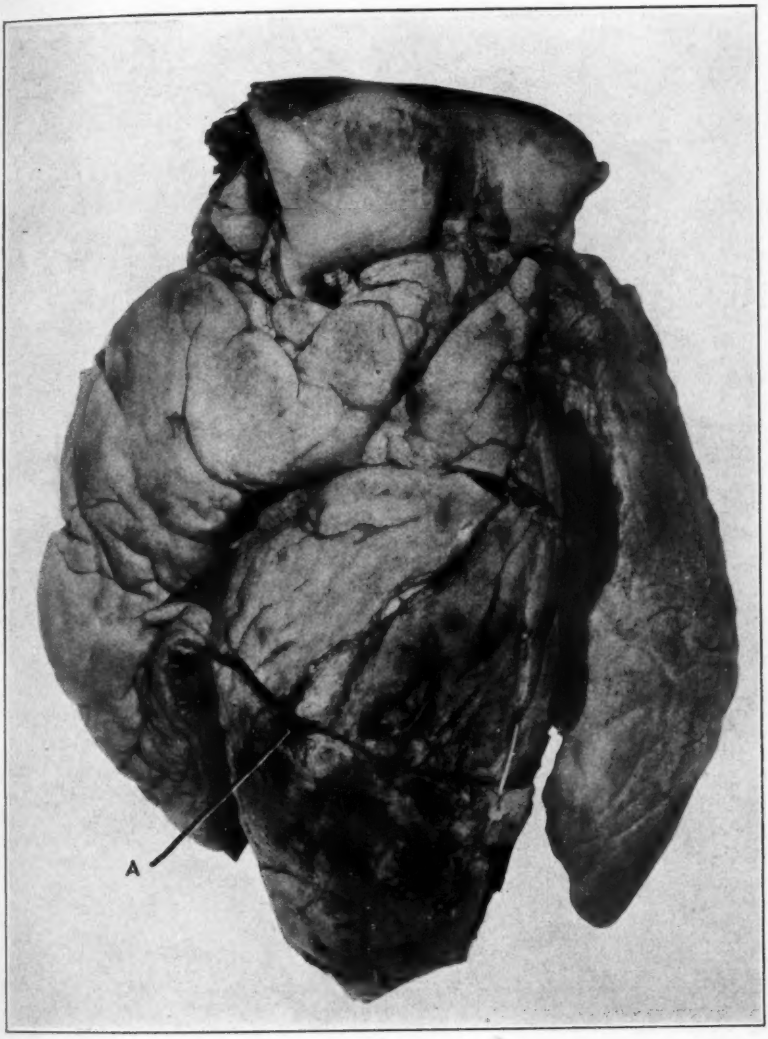
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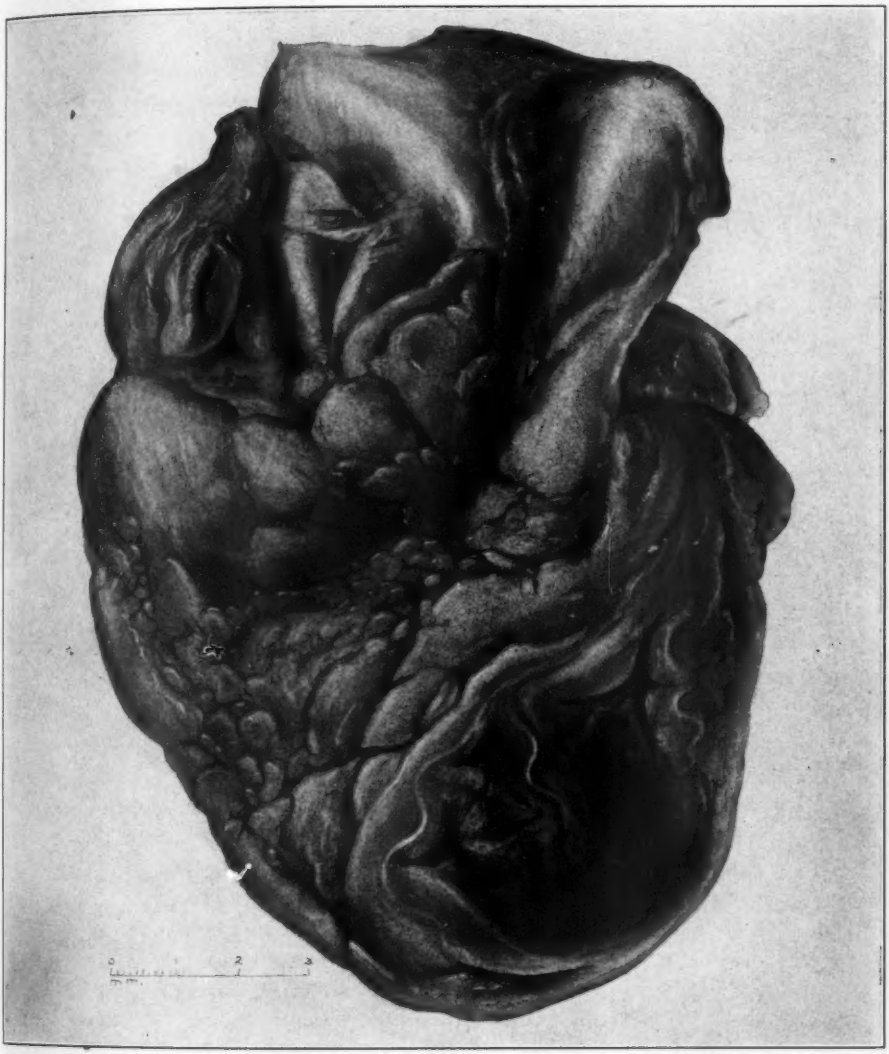
PLATE 66

FIG. 3. Infected infarct of myocardium with hemorrhage and rupture. Coronary thrombo-arteritis with occlusion.



3

THE EFFECT OF THE ORAL ADMINISTRATION OF POTASSIUM IODIDE AND THYROID SUBSTANCE ON THE MITOTIC PROLIFERATION AND STRUCTURE OF ACINI IN THE THYROID GLAND IN GUINEA PIGS*

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In previous papers Loeb has shown that, contrary to the current view, which is that administration of iodine prevents compensatory hypertrophy, this substance does not exert an inhibiting effect, and it may even increase the hypertrophy. Thus Loeb observed in a very extensive series of experiments that the average of hypertrophy was greater in the iodine-fed than in the control animals.[1,2] This finding suggests that the primary effect of iodine on the thyroid gland is stimulating and that when a depressing effect is noticed, it is of a secondary nature; this conclusion applies to the normal thyroid. It appears that if during development of the organism there is a lack of iodine, this lack may also cause proliferation of the thyroid gland and a goitre develops. This proliferation can be prevented through administration of very small amounts of iodine. In certain cases the proliferation which has already begun can even be stopped through administration of iodine. On the other hand, in some instances, administration of iodine to goitrous individuals exerts a stimulating effect, causing proliferative processes and the appearance of toxic symptoms. Loeb concluded therefore that the thyroid tissue may respond to a deficit of iodine, as well as to a surplus of iodine, with increased activity and in particular with growth processes. Apparently the thyroid gland is adapted to a definite amount of iodine which enables it to function normally, and a diminution as well as an increase of iodine above this amount may disturb the normal cell equilibrium and act as a stimulus.[2]

In continuation of these investigations it seemed of interest to study the effect of administration of iodine on the intact thyroid

* Received for publication April 6, 1928.

† We are indebted to Miss F. L. Haven for assistance in these experiments.

gland, in which there is no lack of circulating hormone such as is caused by extirpation of a considerable portion of the gland tissue. Would administration of iodine under these conditions likewise act as a stimulus and call forth proliferative processes? In order to determine the effect in a definite and quantitative manner, we estimated the number of mitoses present at a certain time in the thyroid gland. We found that by this means we could establish in an exact degree the stimulating effect of iodine even in the normal gland.[3] The depression in activity which takes place at a later period and under certain other conditions we attributed largely to pressure effects exerted on the epithelium lining the acini by the material contained in the lumina of the latter; but there may in addition be other factors involved. We have reason for assuming such pressure effects, because previously Loeb has observed in the thyroid of the guinea pig a breaking through of the walls of adjoining acini and a flattening of the lining-cells, evidently under the influence of pressure exerted by the colloid material. Thus the colloid of neighboring acini may form, in the end, one continuous mass; and it is very probable that many of the very large acini, which are found especially in the periphery of the thyroid gland, represent really the result of coalesced acini. It is probable that whole acini may thus disappear as a result of pressure and of consequent interference with the circulation. Similar observations can be made also in cases of human goitrous thyroids and pictures of this kind have been described previously by other observers.[4] Furthermore, differences in the activity in the peripheral, middle and central zones of the thyroid of the guinea pig and the resulting differences in the pressures acting on the acinus cells in these areas are responsible for the development of these three zones of acini which Loeb described in the normal thyroid gland of the guinea pig. The factors mentioned, in particular the pressure exerted on the walls of the acini, explain the relative flatness of the epithelium in the peripheral acini.

In the experiments to be described we compared the number of mitoses found and the structural characteristics of the thyroid gland of normal guinea pigs serving as controls and of KI-fed animals; each of the latter received daily a dose of 0.05 gm. KI by mouth in the form of a pill.* In other experiments we fed a tablet containing

* In feeding the potassium iodide to guinea pigs care must be taken that the animals actually swallow the pill.

0.1 gr. desiccated thyroid gland (Armour & Co.) to the guinea pigs, a different set of animals serving as controls.

We carried out two series of experiments; the first series was made in April, May and early June, 1926; the second in December, 1926, and January and February, 1927, for control and potassium iodide-fed animals, while the experiments with feeding of thyroid gland were completed in April and in the early part of May, 1927. Thus we have to deal with three sets of guinea pigs in each series, namely: (*a*) controls, (*b*) guinea pigs fed with KI, and (*c*) guinea pigs fed with desiccated thyroid gland.

I. The Number of Mitoses

We shall first discuss the results obtained in the second series, because here the method used for the determination of the number of mitoses was more satisfactory than in the first series.

Second Series: Five control guinea pigs, six guinea pigs each fed daily with 0.05 gm. KI and four guinea pigs fed with 0.1 gr. desiccated thyroid gland, were studied. The same kind of food was given to all. At the end of the experiment the thyroid glands were removed, immediately after death by chloroform, and fixed in Zenker's solution. Sections were cut serially.

In every tenth or eleventh section, the mitoses were counted and on this basis the approximate number of mitoses in the whole lobe estimated. In some animals an isthmus was present in the thyroid; it was usually small and not included in our counts.

(*a*) *Control Animals:* The numbers of mitoses found in one lobe of each of the control animals were as follows: 104, 80, 143, 63, 91. Thus an average of 96 mitoses per lobe or of 192 mitoses in the whole thyroid, excluding the isthmus, was obtained (see Table I). This represents the average number of cells which in our experiments were in mitotic division, at a certain time during the winter months, in the normal thyroid gland of guinea pigs, the weight of which varied approximately between 350 and 450 gm.

(*b*) *Potassium Iodide Animals:* Six guinea pigs which were fed with KI daily showed an average of 355 mitoses in one lobe or 710 mitoses in the whole thyroid, excluding the isthmus (see Table I). The lowest count in this series is more than twice as high as the average count in the controls and quite noticeably higher than the highest count in the control series.

(*c*) *Thyroid-Fed Animals:* In four guinea pigs, which were fed with 0.1 gr. thyroid gland daily for from 18 to 30 days, the counts of mitoses were as follows: 45, 0, 42, 40. The average count for one lobe was 32; for the whole thyroid, excluding the isthmus, it was 64 mitoses (see Table I).

First Series: In the first and larger series, the number of mitoses was estimated in a different and less accurate manner than in

Table I

Mitotic Activity in Series II

KI-fed animals			Control animals		Thyroid-fed animals		
Guinea-pig no.	Duration of feeding in days	Mitoses*	Guinea-pig no.	Mitoses*	Guinea-pig no.	Duration of feeding in days	Mitoses*
10	16	868	79(*a*)	208	233	20	90
11	17	1008	79(*b*)	160	270	30	0
12	19	962	78	286	250	18	84
13	21	460	57	126	217	20	80
14	21	390	58	182			
15	23	572					
Averages of mitoses		710		192			64

* Mitoses, per whole gland, excluding isthmus.

the second series. In ten sections of the thyroid of each animal, the mitoses were counted in the central area, characterized by the larger size of the acini and the greater height of the epithelium. These central areas were, on the whole, somewhat smaller in the KI pieces, which were taken out for examination at later periods, than in the controls.

A larger number of sections were used for estimation of the number of mitoses in the thyroid glands of animals fed with thyroid substance than in the others to compensate for the smaller size of the areas in these thyroid glands. While, as stated, this method is less accurate than the method used in the later series, still the results agree fairly well in both and we may therefore consider the results here obtained as corroborative of those obtained in the second series.

(*a*) *Control Animals:* The results obtained are shown in Table II. The average number of mitoses counted in these 10 guinea pigs was 2.4. There were variations between 0 and 7 mitoses in the individual cases. The weight of the animals varied between 328 gm. and 870

TABLE II

Mitotic Activity in Series I

Control animals		
Guinea-pig no.	Weight in grams	Mitoses
134	695	5
218	660	7
104	580	1
345	515	1
458	620	1
332(*a*)	870	1
331	665	0
269	650	1
335	445	3
265	328	4
Average number of mitoses		2.4

gm., and the average weight was approximately 550 gm. The thyroid glands of these guinea pigs were removed for examination during the months of April and May.

(*b*) *Animals Fed with KI:* The average number of mitoses in all the animals in this series which were fed with KI is 6.3. The weight in the KI group varies approximately between 400 and 700 gm. However, there should be omitted from this list Guinea pig 212, which had been fed only during a period of 5 days with KI; the iodine effects were apparently not yet noticeable at this early period. Furthermore, we should omit Guinea pigs 315, 263 and 22 whose thyroids were removed as late as 108 days after the beginning of the

administration of KI. At this time marked retrogressive changes had begun in the thyroid gland. Omitting these four guinea pigs, the average number of mitoses of the remaining 13 guinea pigs is 8.1 (see Table III).

TABLE III

Mitotic Activity in Series I

KI feeding			
Guinea-pig no.	Weight in grams	Duration of feeding in days	Mitoses
212		5	2
216	768–773	8	8
217	865–850	10	7
264	410–423	12	12
267	390–375	15	8
328	520–585	21	15
268	520–547	25	7
386	465–513	30	5
41	485–600	30	1
37	535–580	30	9
306	465–595	40	14
367	525–605	40	6
300	415–485	60	3
330	393–420	60	10
315	735 end weight	108	0
263	650 end weight	108	1
22	815 end weight	108	0
Average number of mitoses (omitting Guinea pigs 212, 315, 263, and 22)			8.1

(*c*) *Animals Fed with Thyroid Gland:* Seven guinea pigs were fed with thyroid substance. The results obtained are presented in Table IV.

The weight in this group varies approximately between 500 and 600 gm. If we omit Guinea pig 420 in which, after only five days of feeding, the full effect of the thyroid substance had evidently not

TABLE IV

Mitotic Activity in Series I

Thyroid feeding			
Guinea-pig no.	Weight in grams	Duration of feeding in days	Mitoses
420	565–502	5	6
474	618–595	8	3
484	538–615	10	1
246	510 end weight	30	2
247		35	0
156	525–543	43	0
133	630 end weight	48	1
Average number of mitoses (omitting Guinea pig 420)			1.16

yet become apparent, the average number of mitoses in this group is 1.16.

Although the absolute number of mitoses is less accurately determined in the first series, it is of interest to compare the proportion in the number of mitoses in the controls, KI- and thyroid-fed animals in the first and second series. For this purpose we consider the number of mitoses in the thyroid-fed series and refer the number of mitoses in the other series to this unit number. In the first series, the proportions are as follows: Thyroid-fed group = 1; Controls = 2.1; KI group = 7. In the second series the proportions are: Thyroid-fed group = 1; Controls = 3; KI group = 11.

While the proportions found in Series II are more accurate than those obtained in the first series on the whole, the results in

both series agree. The number of mitoses is about three and a half times as great in the KI group as in the control group, and two to three times as great in the controls as in the thyroid-fed group. We may then conclude that during the first three to four weeks, and possibly even somewhat longer, KI feeding increases definitely and considerably the mitotic activity of the thyroid gland, and that on the contrary feeding with thyroid gland diminishes the number of mitoses (see Figs. 1 and 2).

II. The Size of the Acini and of the Cells Lining them and the Character of the Colloid

(*a*) *Control Guinea Pigs:* In comparing the acini, the character of the acinus cells and of the colloid in the three series we must take into consideration the fact that noticeable variations may occur even in the control animals. Within the same group of animals the acini and the acinus cells present considerable differences in size. In the average control thyroid gland the acinus cells were medium to low, as far as their height was concerned. The colloid was slightly retracted; there were many vacuoles in the peripheral parts of the colloid; it was of medium consistency. The variations ran from large and medium-sized acini with low epithelium and with harder colloid that was slightly retracted, to acini of variable size, irregular in contour with relatively high epithelium and with colloid that was adherent to the epithelium in various places and separated from the latter merely by vacuoles.

(*b*) *Guinea Pigs Fed with KI:* If we compare the structure of the thyroid glands of guinea pigs fed with KI with the condition found in control guinea pigs, we find, during a period of approximately the first four weeks following the beginning of the experiment, no very decided differences between these two groups of animals. Again considerable variations in individual cases are present; while in some animals the average height of the acini cells may perhaps be less than in the controls, in others it is equal in the two groups, and in some cases it is even greater in the KI group. In particular, the thyroid of Guinea pig 10 fed with KI for a period of 16 days showed not only a relatively high epithelium but in addition colloid which, on the whole, was very soft and partly liquefied. This gland resembled the thyroids found in some cases of compensatory hypertrophy.

The thyroid of Guinea pig 12, examined after 19 days of daily KI feeding, showed acini with epithelial cells which were slightly higher than medium-sized; some of the acini had prominences extending into the luminae and were irregular in outline. The colloid varied somewhat in different acini; it was fairly hard in some, softer and even partly dissolved in others. In a number of acini it could be shown that the solution was produced, at least in part, through the activity of cells which phagocytosed particles of the colloid. The thyroid of Guinea pig 15, after a period of KI feeding extending over 23 days, was similar, although on the whole, the colloid was here somewhat harder and slightly retracted. Figure 3 (Guinea pig 328) shows the structure of the gland after a period of 21 days during which the animal received a daily dose of KI. The epithelium is of medium height in the majority of the acini, but in a number of the larger acini it is somewhat flatter evidently owing to pressure exerted by the contents of the acini on the layer of epithelial cells. The colloid appears, on the whole, soft and in a number of acini it has been invaded by phagocytic cells which have almost destroyed it.

These examples may serve to show that, for the most part, there is no marked difference in the size and character of the epithelium and of the acini between the control and KI guinea pigs within the first four weeks following the beginning of the KI feeding.

If we now consider the later stages of iodine feeding, we may state in general that there is a tendency for the acinus cells to become low; at the same time the colloid may be solid and be present in the acini in a relatively large quantity, but in other cases solution processes have taken place in it. There is, in addition, a tendency for the acini to become larger. These characteristics are quite definite in the specimens examined 108 days after the beginning of the feeding with KI. Fig. 5 (Guinea pig 315, fed with KI during a period of 108 days) illustrates this stage very clearly. The acini here are large, the epithelium is very low and the walls are thin. What is left of the colloid is very markedly retracted, much more so than in the earlier stages or in the control specimens. This marked retraction is probably a result of the action of fluid on the colloid and indicates softening processes which have taken place in this substance in certain of the acini. There is very little doubt, however, that pressure has been exerted on the walls of the acini by the colloid, or by the substances produced from the colloid through solution processes, and that this

pressure is largely responsible for the greater size of the acini and for the flatness of the epithelium at this period. In addition, we may assume that such an increase in pressure will lead to an interference with the circulation in the spaces between the acini. As a result of the increased intra-acinar pressure, which exists in the later periods of KI feeding, we find here and there that the walls of adjoining acini are flattened and broken through. Thus smaller acini may be united forming larger ones and, as Loeb has stated previously, the very large acini have been produced in many cases as the result of this secondary union of adjoining acini. The colloid of such acini may at first be united by a bridge passing through the openings in their walls, but it may finally form one connected mass. A transition to this condition is found in Fig. 4 (Guinea pig 37), where as early as 30 days after beginning the KI feeding some of the typical effects of the later periods of iodine on the thyroid gland are becoming noticeable. The acini are large, the colloid is correspondingly great in quantity, but still rather solid. The acinus cells, while on the whole fairly low, are distinctly higher than at later stages. The colloid here does not show the marked retraction seen in Fig. 5; in various places it still adheres to the wall of the acinus.

(*c*) *Effect of Thyroid Feeding on the Structure of the Thyroid Gland:* In the first week of thyroid feeding no definite differences were seen between the structure of normal glands and the glands of the thyroid-fed animals. From the tenth day on, there was on the whole, perhaps, a tendency on the part of the acinus cells to become somewhat flatter and for the colloid to be rather solid; the acini were, on the average, probably somewhat smaller than in the controls. However, these differences as a rule were not very pronounced and not present in all cases. The latest term at which the gland of a thyroid-fed guinea pig was examined by us was 48 days.

We shall cite as an example, the findings in Guinea pig 247, examined after 35 days of thyroid feeding. The acinus cells are here very low; the colloid is hard, almost entirely detached from the wall and rather markedly retracted. In some places the walls separating two adjoining acini have become very thin and they have the appearance of being ready to break through. Similar pictures of the rupture of walls of neighboring acini occur in thyroid glands in other instances and even in the thyroids of control animals, also wherever there is pressure exerted on the tissue separating two acini; this

process is therefore associated with flatness of acinus cells in the area in which such changes are taking place. The majority of the acini represented in this example are still relatively large, and in this respect the picture does not perhaps represent the average findings in the glands of thyroid-fed guinea pigs.

III. On Certain Secondary Changes in the Thyroid Gland

In a number of cases we found small accumulations of lymphocytes in the stroma of the thyroid gland. Furthermore, not infrequently, we observed phagocytic cells in the colloid of acini; these take up small particles of colloid into their cell bodies and here destroy it; as a result of this process the colloid may assume a honeycombed appearance. Colloid was also seen, in certain cases, in the interstitial connective tissue that separates the acini. The latter condition seems to depend on the destruction of the walls of some acini, perhaps as the result of pressure, and the subsequent escape of colloid into the interstices of the stroma. However, in interpreting such pictures, we must be aware of the possibility that colloid may be artificially squeezed out from the acini into the connective tissue spaces and we have to distinguish between these two occurrences.

If we now compare the three groups of guinea pigs as to occurrence and frequency of these changes, we find that the latter condition may be seen in all of them; the phagocytic activity, however, seems to be more pronounced in the KI-fed animals than in the controls.

Discussion

Our experiments have thus shown that in the early stages of administration of iodine to normal guinea pigs, the number of mitoses is quite noticeably increased in the acinus cells of the thyroid gland and we may therefore conclude that iodine exerts primarily a stimulating effect on this gland. While we believe that the number of glands which we have examined is sufficiently large and that the results are sufficiently concordant to justify a general conclusion concerning this formative stimulation exerted by iodine in the case of the guinea pig, still we do not consider our observations will permit us to draw definite conclusions, as yet, as to the intensity of the

increase in cell proliferation, which we find here as the result of iodine feeding, or as to the average number of mitoses found in normal thyroids of guinea pigs. Further investigations, which are being carried out in our laboratory at the present time, may be expected to determine these conditions more definitely.

Of special interest in our investigations is the difference which we found between the action on the thyroid gland of iodine feeding and of the feeding with thyroid gland. Our observations in this respect correspond to the differences established in the influence exerted by these two substances in compensatory hypertrophy of this gland where feeding with thyroid (and to a lesser extent also with anterior pituitary gland) depresses, whereas feeding with KI stimulates, the proliferative processes in the acini. We may explain the difference between the action of these two substances in the following way: Feeding with thyroid gland introduces an excess of the hormone into the circulation and produces the effects observed in the thyroid gland of the animal. Such effects are the opposite of those which follow operative removal of a considerable portion of the thyroid gland tissue; the latter procedure leads to a diminution in the circulating hormone and stimulates growth processes in the remaining part of the thyroid gland; the former procedure depresses the activity of the gland, the function of which is now no longer needed and which might even become injurious. On the other hand, the administration of iodine is not identical with the introduction of ready-made hormone; in the former instance a substance has been introduced which is able to initiate the production of hormone in the gland cells, and it is apparently this stimulating process, set in motion by iodine, which also induces the gland to proliferate; this phase of action is necessarily lacking if we introduce the hormone as such. In a subsequent phase, when possibly an excess of hormone has been produced as a result of this stimulation of the thyroid gland, there may perhaps be added to the primary stimulation exerted by iodine, a secondary inhibiting effect due to the presence of an excess of hormone which may thus, secondarily, cause a depression in the activity of the gland.

However, whether this secondary inhibiting effect of hormone action comes into play at a certain stage and thus causes a depressive action we must consider problematical at the present time. On the other hand, there are definite indications that pressure is exerted

on the walls of the acini which produces the flattening of the epithelial cells, the enlargement of the acini, and in many cases the perforation of the wall of adjoining acini. And we may attribute to this factor, at least in part, the decrease in activity found in later stages of KI administration. This pressure is due to the action of the unchanged or partially liquefied colloid which is not removed from the acini of the gland in the same active manner in case of KI feeding, as it is in the condition found in compensatory hypertrophy where the removal of thyroid gland tissue, in some way, leads to a mobilization of the colloid and where thus neither the colloid as such nor the substance into which the colloid is secondarily transformed is able to the same extent to exert pressure on the inner lining of the acini.

The results formerly obtained by one of us concerning the effect of iodine on compensatory hypertrophy of the thyroid gland and his conclusions as to the primarily stimulating effect of iodine on this organ, as well as our present results regarding the effect of iodine on the normal gland, are at variance with the widely accepted view that iodine in general prevents proliferative changes in the thyroid gland and produces a quiescent condition of this organ. According to Marine and his collaborator,[5] after once a goitrous proliferation and metabolic hyperactivity have started in the thyroid gland, administration of iodine leads to the production of an increased amount of colloid in the acini and thus ultimately a colloid goitre is produced, which under these conditions represents the ultimate resting stage of the abnormal gland.

Summary

1. Oral administration of iodine to guinea pigs markedly increases the mitotic activity in the thyroid gland during the first three to four weeks.

2. This first phase of increased activity is followed by a second phase of depression, which is accompanied by definite structural alterations in the thyroid gland. We find indications that this second phase is at least partly due to pressure exerted by the contents on the walls of the acini.

3. In contradistinction to the effect of potassium iodide, administration of thyroid gland substance is not followed by a phase of

stimulation, but in this case, within the first two weeks, a period of depression sets in which may be accompanied by certain structural changes in the gland. As the result of thyroid feeding the mitotic activity is diminished as compared with that found in the controls.

REFERENCES

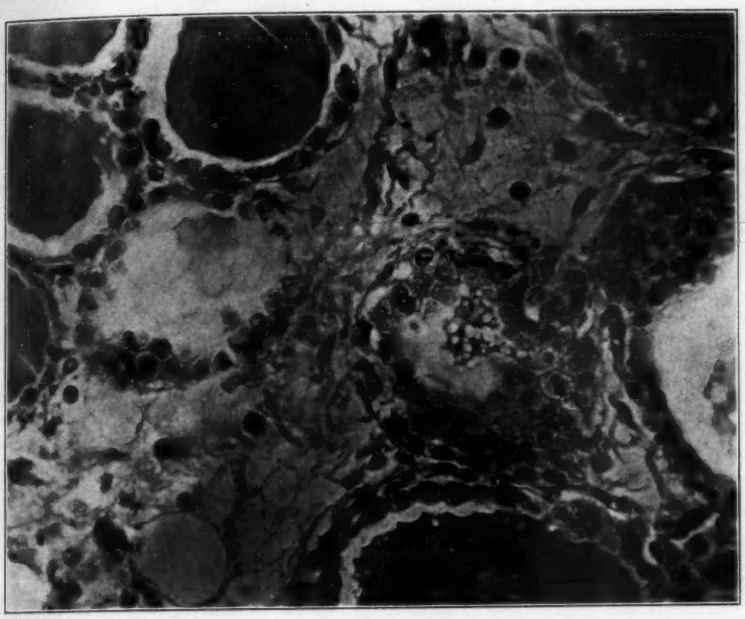
1. Loeb, Leo. *Am. J. Path.*, 1926, ii, 19; *J. Med. Res.*, 1920, xli, 481.
Loeb, Leo, and Kaplan, E. E. *J. Med. Res.*, 1924, xliv, 557.
2. Loeb, Leo. *J. Med. Res.*, 1920, xlii, 77.
3. Gray, S. H., Haven, F. L., and Loeb, Leo. *Proc. Soc. Exper. Biol. & Med.*, 1927, xxiv, 503.
4. Reinhoff, W. F. *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 285.
Cattell, R. B. *Boston M. & S. J.*, 1925, cxcii, 989.
5. Marine, D., and Lenhart, C. H. *Arch. Int. Med.*, 1909, iv, 253.
Marine, D. *Arch. Path. & Lab. Med.*, 1926, ii, 829.

DESCRIPTION OF PLATES

PLATE 67

FIG. 1. Guinea pig 11. 17 days KI feeding. Central part of gland, showing two mitotic figures, tall epithelium, softening of colloid.

FIG. 2. Guinea pig 11. 17 days KI feeding. Periphery of gland, showing two mitotic figures, one in center slightly out of focus. The epithelium is fairly high.



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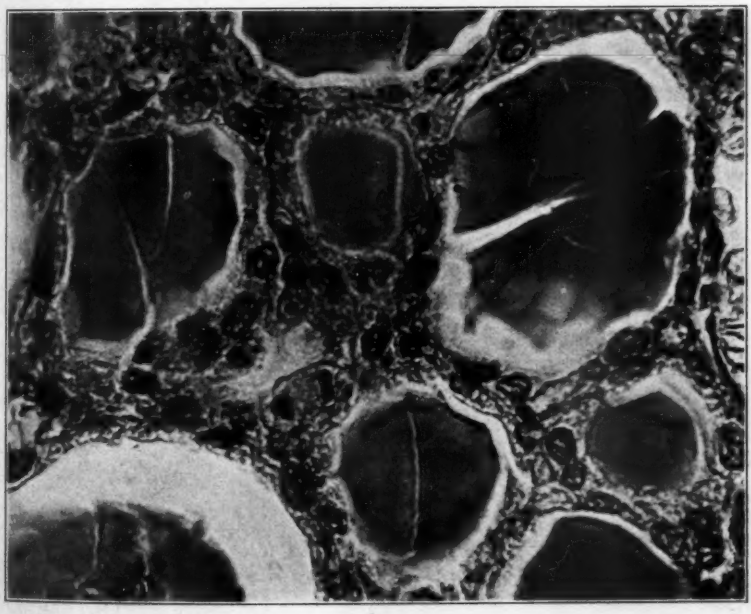


2

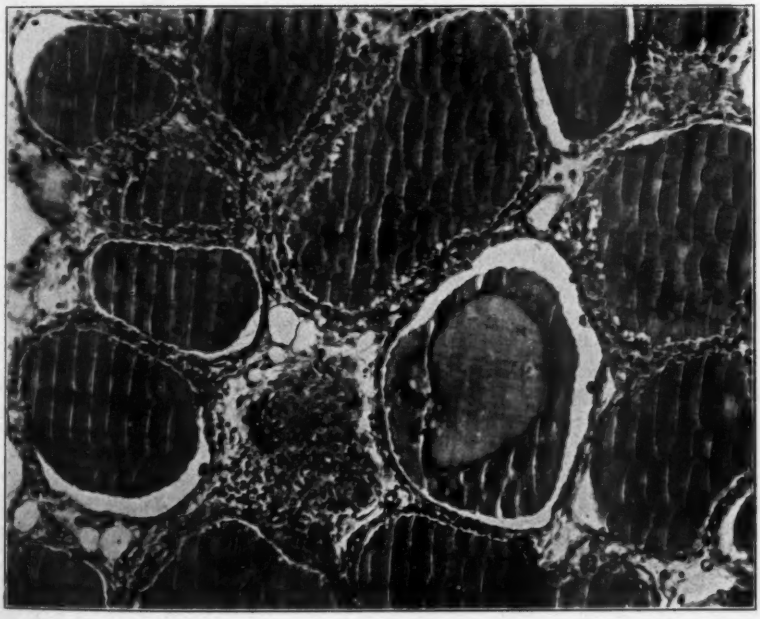
Gray and Loeb Thyroid Gland

PLATE 68

FIG. 3. Guinea pig 328. 21 days KI feeding.

FIG. 4. Guinea pig 37. 30 days KI feeding. Beginning change to low epithelium, hard colloid.

3



4

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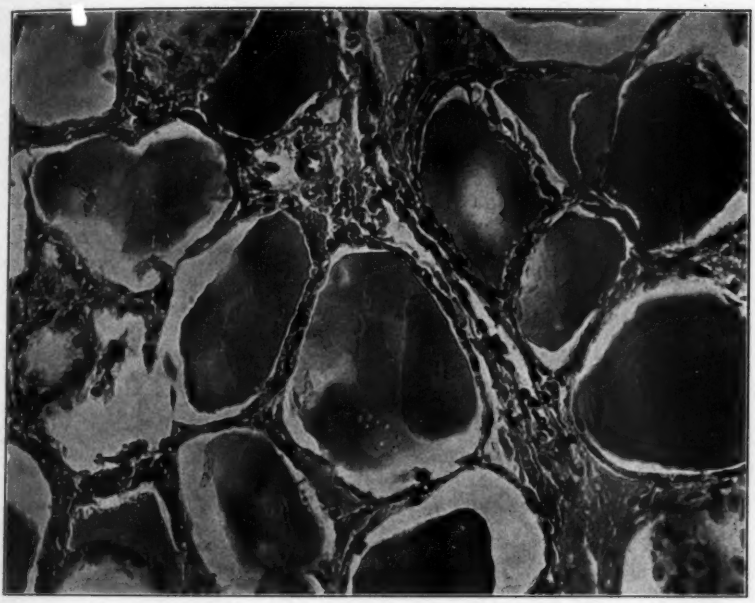
PLATE 69

FIG. 5. Guinea pig 315. 108 days KI feeding. Flat epithelium, soft colloid. In the place reproduced in this picture the acini were smaller than elsewhere in this gland.



5

Gray and Loeb Thyroid Gland

OBSERVATIONS ON INCUBATED NORMAL BLOODS *

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The past five years have witnessed an increasing interest in the cells of the blood. The modern methods of treating the anemias and the conception that there may be a cell in the blood stream which reacts specifically to certain infections, such as tuberculosis, have stimulated a number of workers to investigations on the subject. The cell which has received particular attention is the so-called monocyte or large mononuclear cell of Ehrlich. This is probably because there is no unanimous agreement as to the classification, origin and physiologic activities of this cell. One method of attack on this problem has been the use of cultures of blood and of tissues in the hope that by observing the development and activity of the cells a proper classification could be arranged.

In recently published observations on cultures of leukemic blood [1] we reported the development of two types of large cells. The question of the appearance of similar cells in cultures of normal blood was considered a matter of sufficient interest to warrant a series of experiments. The object of this work was to determine if the monocyte of the blood developed into an actively phagocytic large cell resembling the tissue phagocyte.

To make the discussion clear it is necessary to define the terms which are used in this paper.

Monocyte

This cell is the one called the large mononuclear or transitional cell by Ehrlich; monocyte by Nageli and Sabin, Cunningham and Doan; and endothelial leucocyte by Mallory and his school. Stained by Wright's method this cell shows an oval or indented nucleus somewhat eccentrically placed, which contains less chromatin and that more scattered than the chromatin in the nucleus of a lymphocyte. There is a considerable amount of cytoplasm which has a

* Received for publication April 6, 1928.

peculiar fine, faint, uniform neutrophilic granulation. The supravital spread method of studying the cells of the blood stained by neutral red and Janus green which was originated by Pappenheim [2] and brought into general use by Cunningham, Sabin and Doan [3] has aided the study of these cells. The nucleus is mottled when observed in this manner and does not contain a nucleolus. The centrosomes located near the center of the cell are usually surrounded by mitochondria and granules which stain with neutral red. This grouping of red stained granules is called a rosette by Sabin and her coworkers. They state that this type of cell never phagocytes material in the central region where the rosette is found but always at the periphery. They consider the phagocytic activity of this cell to be definitely limited as compared to that of others such as the clasmatocyte. The monocyte is actively motile though less so than the polymorphonuclear leucocyte. It has thin pseudopods and more cytoplasm in proportion to nuclear material than the lymphocyte.

Lymphocyte

This term includes all the mononuclear cells of the blood except the monocytes. Stained by Wright's stain the nucleus is round or sometimes indented. There is a large amount of dense chromatin. The cytoplasm may be very variable in amount as compared to the nucleus. The color of the cytoplasm is a clear, light blue. A few azure granules may be present and occasionally a perinuclear clear zone is observed.

Clasmatocyte

This cell is the one called clasmatocyte by Ranvier, adventitial cell by Marchand, pyrrol cell by Goldman, polyblast by Maximow, macrophage by Metchnikoff, and histiocyte by Aschoff and Kiyono. It is a highly phagocytic, actively migratory cell found scattered throughout the connective tissue of the body. Wright's stain brings out a round or indented nucleus with considerable chromatin and a large amount of evenly stained blue cytoplasm. When observed by the supravital technic these cells show the cytoplasm packed with material of irregular size and distribution which stains with neutral red. The nucleus is round, oval or indented, often eccentrically placed and usually contains a nucleolus. Sabin states that this type

of cell contains no mitochondria. Lewis,[4] however, found when films were stained only with Janus green all these cells contained mitochondria but when neutral red and Janus green were combined the heavy mass of red-staining material obscured them. This cell is actively motile and phagocytic. The tendency of the cytoplasm to form pseudopods is very marked.

Culture Data

The first cultures of human blood are those described by Awrorow and Timofejewskij [5] in 1914. They cultivated the blood of patients with myelogenous leukemia. They found that the myeloblasts gave rise to giant cells, clasmatocytes, multipolar cells and cells similar to Maximow's polyblasts. In similar experiments on normal blood, no such cells developed. Therefore, they concluded that the presence of myeloblasts was necessary for the development of the large types of cells.

Timofejewskij and Benewolenskaja [6] in 1927 reported cultures of the blood of a myelogenous leukemia patient who had eighty-four per cent myeloblasts in the blood. They felt that the myeloblasts gave rise to polyblasts, giant cells, clasmatocytes, fibroblasts, granulocytes and probably lymphocytes. In this paper they mention cultures of normal rabbit blood. In these, they described polyblasts, giant cells and clasmatocytes as arising from the monocytes and lymphocytes.

The first successful cultivation of cells of normal blood was that of Carrel and Ebeling [7] in 1922. These workers coagulated the buffy layer of centrifuged blood by the addition of a few drops of embryonic juice. The resulting clot was handled like a tissue culture and the cells examined from time to time. They state that the polymorphonuclears and lymphocytes disappeared after the first week of incubation. Large ameboid cells developed which when stained by neutral red showed variable-sized red granules almost filling the cytoplasm. This cell was considered to be a clasmatocyte. They mention the appearance of a cell of irregular shape, tending to be elongated, which contained very much finer granules staining with neutral red than the clasmotocyte type. They considered this cell to be a fibroblast because groups would occasionally join to form a syncitium. These cells were found to have the same resistance as

fibroblasts [8] to certain toxic agents, such as arsenic. No fibroglia or collagen fibrils were demonstrated.

In a study of the blood and subcutaneous tissues of the chicken these authors [9] concluded that the monocyte of the blood and the clasmatocyte of the tissues were identical cells. Their variation in function and morphology depends upon environmental conditions, not on any difference in origin. They felt that one type could be changed into the other by altering the conditions under which growth took place.

Maximow has claimed since 1902 that the polyblasts or mononuclear cells which appear in inflammation arise in part from local cells, the resting wandering cells or histiocytes of the tissue, and in part from lymphocytes and monocytes which have migrated from the blood vessels. In 1924 [10] he described cultivation experiments using animal tissue. He observed the appearance of large, ameboid phagocytic cells and felt that he could trace their development from tissue histiocytes, blood monocytes and lymphocytes. In later experiments described in 1927 Maximow [11] cultured the leucocytic coat obtained from guinea-pig blood. Lymphocytes were seen to change in shape and size. Active ameboid movements with the development of pseudopods appeared and fat droplets were present in the cytoplasm. The monocytes were also seen to hypertrophy. He states that by the second or third day monocytes and lymphoctyes indiscriminately had transformed themselves into large phagocytic polyblasts.

Fischer [12] in 1925 followed the technic of Carrel and cultivated a clotted buffy coat of centrifuged blood. Muscle, which had been kept in the ice box until the fibroblasts were no longer viable, was added to the culture. Pure cultures of cells were obtained, similar to those described by Carrel and Ebeling. Many granules of neutral red were seen in the cytoplasm. As the outline of these cells was irregular and elongated they were considered to be fibroblasts.

In 1924 and 1925 M. R. and W. H. Lewis, [13, 14, 15, 16] together and separately, carried out extensive observations on the blood of a large number of species of animals. Their method was to allow a drop of blood to clot on a coverslip which was inverted over a hollow ground slide and sealed with petrolatum. The cultures were incubated at 37.5° C or kept at room temperature when they were from cold-blooded animals. To examine such specimens the plasma drop was

washed off with Locke's solution to which neutral red or Janus green had been added. Such preparations had to be discarded after study.

These workers attempted to follow the changes occurring in the various types of cells seen in the living blood cultures in order to determine just which cells were transformed into macrophages, epithelioid cells and giant cells. As controls, fresh cultures were examined shortly after their preparation in order to identify the various types of white cells with and without the aid of neutral red and Janus green, and to determine whether or not macrophages, epithelioid cells and giant cells were ever present in the normal blood. Such cells were never found in the first few hours. Though no special effort was made to determine how long the various types of cells lived and no sub-cultures were made the cells sometimes remained alive in the primary cultures three to four weeks. Very little change was seen in the granulocytes cultivated by this method. They developed a few neutral red-staining granules and occasionally phagocyted red cells. The lymphocytes showed an absolute increase in the number of neutral red-staining granules. Although any direct transformation of lymphocytes into mononuclears was not seen these authors state that the many intermediate stages between the two forms suggested that possibly such a transformation occurred. The monocytes were seen to become modified into macrophages, epithelioid cells and various intermediate forms. These workers describe mononuclears which showed active phagocytosis of red blood cells. Other mononuclears showed little phagocytosis but accumulated a large number of small neutral red-staining granules about the centrosome. These structures gradually became larger until they almost filled the cell. The mononuclears increased in size and often fused to form giant cells. They frequently were irregular in shape and showed long pseudopods.

These workers concluded from their long series of experiments that mononuclears, macrophages and epithelioid cells are merely different phases of the same cell type.

The work of two other investigators should be considered in this connection. Kindwall [17] presented evidence that monocytes were not present in lymph from the thoracic duct. Bloom [18] made clot cultures of thoracic duct lymph and observed the cells which developed by both supravital spreads and fixed, sectioned preparations. He saw the appearance of large cells containing a well marked

rosette when stained with neutral red. There were numerous transition forms between these cells and lymphocytes. The large cells showed many granules staining with neutral red and some lipoid droplets in the cytoplasm. They were actively phagocytic and a certain number of long spindle-shaped cells appeared. When fixed and stained they showed a basophilic cytoplasm and a larger, more vesicular nucleus than the lymphocytes.

Methods

The methods described below were usual in our work. The use of plasma clot cultures makes repeated observations on the same preparation impossible. By employing a fluid culture medium we have been able to follow the same cultures for long periods.

Blood was drawn under aseptic precautions from the median basilic vein of various workers in the laboratory. Rabbits were bled from the heart. About 1 cc. of blood was placed before clotting in small test tubes. In some cases 2 cc. of blood were added to 1 cc. of a 1:200 dilution of heparin in Locke's solution giving a final heparin dilution of 1:600. Occasionally 1 cc. of the blood-heparin mixture was diluted with 9 cc. of Locke's solution. Some of the rabbit blood was mixed with one per cent sodium citrate in equal proportions: 2 drops of this mixture were added to 1 cc. of rabbit serum. Where a culture of clotted blood was desired the tube was ringed with a sterile loop as soon as a moderately firm clot had formed. The tubes were sealed with paraffin and incubated at 37.5° C. In some cases control tubes were kept in the ice box. Particular care was used to keep the tubes away from the light. Observations were made from time to time by removing a drop of sediment from the bottom of the tube with a platinum loop. This method permitted repeated observations to be made of the same culture.

Each sample was observed in a supravital spread and air-dried smears were usually made at the same time. Most of the supravital slides were prepared with neutral red only as the toxicity of Janus green prohibited its routine use. Observations with Janus green were made whenever there was any question as to the presence or absence of mitochondria. The dried smears were stained by Wright's blood stain and by the method of Sato and Sekiya [19] for the oxidase reaction. A few of the clot preparations were fixed in Zenker's fluid,

embedded in paraffin, sectioned, and stained with eosin-methylene blue. Occasionally preparations which had been stained supravitally were pulled apart and stained by Wright's method. Supravital observations on preparations which had been in the warm box more than thirty minutes were considered invalid.

The presence or absence of hemolysis was found to be good evidence as to the presence of living cells. If the tube showed no hemolysis it was fairly safe to prophesy that no living cells would be found.

Rabbit's Blood

Granulocytes: These cells could be followed with little difficulty until the sixth day and sometimes longer. From the second day on they became progressively less motile and exhibited neutral red granules of irregular size and a few large unstained refractile granules in the cytoplasm. Usually from the fourth to the sixth day it was very difficult to identify them positively. By the sixth day nearly all were rounded up and unstained. The dead forms could frequently be seen in the Wright's stained preparations at the end of two weeks incubation. The oxidase reaction remained positive long after the death of the cells. The eosinophiles persisted almost unchanged for weeks.

Lymphocytes: The small lymphocytes showed very little change during a long period of incubation. They could usually be followed to the second week and sometimes longer. The tendency to develop an increased number of granules staining with neutral red as described by Lewis was not particularly marked. Transition forms between small lymphocytes and monocytes were never seen. The morphology remained constant as observed in the fixed preparations.

Monocytes: During the first three days the neutral red-staining rosette of these cells became more definite and larger as the granules increased in size and number. After twenty-four hours, uniform, round unstained granules began to appear in the cytoplasm. These granules increased in number as the time of incubation went on. They tended to be arranged at the periphery of the cell. They reacted for neutral fat with Nile blue sulfate. By the fourth day these cells were of good size and phagocytosis was quite active but variable. From that time on the cells increased in size until a maximum diameter of about twenty-five to forty microns was attained.

A rosette of good sized, red-staining granules near the nucleus persisted and often the cytoplasm was completely filled with unstained refractile granules. Stained by Wright's method these cells showed a round to oval nucleus, fairly vesicular in type. The cytoplasm was blue in color and contained a large number of unstained vacuoles.

X Cells: At about the fourth day a type of cell appeared which was somewhat different from any seen in the blood up to that time. Observed by the supravital technique the cytoplasm contained a large number of fine red granules suggesting the hypertrophied rosette mentioned by other workers. Refractile granules were present occasionally but were fewer in number and finer than those seen in the cells described as monocytes. Phagocytosis was very active; the cytoplasm often contained great masses of red-staining material and cells. Multinucleated forms were frequently seen. The outline of these cells was quite irregular. Many stellate and elongated forms were present and the tendency to form pseudopods was well marked. Motility usually persisted until the end of the second week when most of the cells became quite sluggish and tended to be round. Stained by Wright's method the nuclei appeared round and contained a fair amount of chromatin. There was a considerable amount of even blue cytoplasm. Some basophilic granulation was present occasionally and often masses of red-staining material. The oxidase reaction was both positive and negative.

As far as could be determined there was no consistent difference according to the conditions under which the cells were kept. There was a suggestion that the cells in the tubes diluted with Locke's solution changed more slowly than those in the clotted tubes. The same types of cells were found irrespective of the method used.

Bloods kept in the ice box showed none of the changes exhibited by those in the incubated specimens. The cells remained alive and active until about the fourth week.

Human Bloods

Granulocytes: Observed by the supravital method the neutrophiles contained an increasing number of red-staining masses and a few unstained refractile granules in their cytoplasm after the first day. They gradually became less motile and more distorted in shape.

By the fifth day they were so changed that recognition was impossible and nearly all were dead. Only a rare one was seen after this time. Stained by Wright's method they could be recognized for two weeks but were extremely misshapen. The oxidase reaction persisted long after the death of the cells.

The eosinophiles showed almost no change and could be followed sometimes as long as two weeks.

Lymphocytes: The small lymphocytes persisted in a living state as observed by the supravital spread method until about the tenth day. There was a slight tendency for the red granules to increase in size and intensity of staining as the period of incubation was prolonged. After the tenth day the outline of the cells could be seen but they were considered to be dead as indicated by the lack of motility and of red-staining granules. In the Wright's stained preparations they could be followed unchanged for two weeks. There was practically no variation in morphology. The oxidase reaction was consistently negative.

Monocytes: The changes which took place in this type of cell were followed from the first two hours of incubation to the death of the cell. In some cases daily observations were made. There was usually little question as to the identification of the monocytes and their derivatives. By the end of twenty-four hours incubation many had developed a variable number of round, unstained refractile granules. The focus of neutral red particles making up the rosette became somewhat larger and the granules more numerous as time went on, but the diffuse, finely granular, hypertrophied rosette was not seen in this cell. The focus of red granules usually persisted until the death of the cell. The cells became gradually larger by phagocyting material which stained with neutral red and by the development of more refractile granules. These stained like neutral fat with Nile blue sulfate. By the sixth to the eighth day the characteristic picture was the presence of a variable number of cells measuring from ten to forty microns in diameter. The nucleus was indented and contained no nucleolus. More than one nucleus was rarely observed. A focus of rather coarse red granules was usually present. The remainder of the cytoplasm was filled with fairly uniform, clear, unstained granules among which globules of red-staining material were scattered. Motility was sluggish at all times but was observed as late as the tenth day of incubation. The cell outline

tended to remain quite rounded; true elongated and stellate-shaped cells of this type were not observed.

Stained by Wright's method these cells had a very characteristic appearance. They stood out sharply against the background of granular débris on account of the unstained vacuoles. Every transition could be observed between the twenty-four hour old monocyte with a few unstained granules and the large cell seen after six or more days of incubation with the rosette persisting and a cytoplasm honeycombed with unstained areas. No transitions were seen between the monocyte and any other type of cell seen in the cultures. When the oxidase reaction was applied to these cells it was usually positive.

In discussing this type of cell later in the paper we will speak of it as the Y cell.

X cells: From the third day of incubation on, a cell appeared which was unlike any seen in the blood before incubation. When first seen it was actively motile and possessed an oval nucleus often with a nucleolus. The number of nuclei varied from one to four. The cytoplasm was much more irregular in shape than that of the monocytes. Stellate and tremendously elongated forms were observed. The cytoplasm contained a large number of fine, uniform, somewhat brown-red granules which were concentrated near the nucleus in a rather dense mass suggesting a rosette. The granules of this structure were consistently finer and more evenly distributed than were those of the monocyte. Phagocytosis was very active and sometimes the whole shape of the cell was distorted by great masses of phagocyted material. Refractile unstained granules were rarely present and if seen were very fine, of irregular size and few in number. As the period of incubation increased this type of cell became increasingly larger, in many cases attaining a diameter of sixty to seventy microns. Forms containing from two to five nuclei were observed and coalescence of single cells of this type to form giant cells could often be watched.

In the dried smears stained by Wright's method these cells could be seen very clearly. There was a round to oval nucleus with fairly dense chromatin and a variable amount of clear blue cytoplasm. As incubation continued, these cells took up a large amount of phagocyted material in their cytoplasm. Red and white blood cells and irregular masses of hemoglobin could be seen. They often dis-

played a few basophilic granules near the nucleus. Multinucleated giant cells were common. As time went on these cells took up more and more foreign material until at the end of the second week of incubation little was seen but a great mass of red-staining material and a nucleus. Transition forms between this type of cell and the monocytes were not seen. This cell is often negative to the oxidase reaction but not infrequently one containing an oxidase-reacting granule was seen.

Discussion

From the foregoing data we may conclude that large phagocytic cells develop in incubated rabbit and human blood. Two types of these cells were seen. From observations made at different periods of incubation it is possible to make some deductions as to the source of such large cells. No change was seen in the small lymphocytes or the granulocytes which would lead one to believe that they ever increased in size and became phagocytic. The only remaining possibilities are the blood monocyte or some other type of cell existing normally in the blood stream. Such cells have been described by Ferrata as the hemohistioblast, by Maximow as the hemocytoblast and by Sabin as the clasmatocyte. The blood monocytes could be followed accurately from day to day. They retained their small, distinct rosettes although the granules increased somewhat in size and number. Refractile granules which showed as unstained vacuoles in the Wright's stained preparations appeared at the end of twenty-four hours and increased in size and number thereafter. Phagocytosis was not particularly active and multinucleated cells of this type were not often seen. The oxidase reaction was nearly always positive.

The second type of large phagocytic cell appeared about the third or fourth day of incubation. When first seen it was very actively motile with long pseudopods. The size was that of a blood monocyte or somewhat larger. Phagocytosis was very active. Observed in supravital spreads the cytoplasm contained a large number of very fine, diffuse red granules, sometimes tending to group in one area, suggesting the hypertrophied rosette described by some workers, and sometimes quite uniformly distributed throughout. Multinucleated forms were often seen. Refractile granules were fewer and smaller than those seen in the monocyte derivatives. When stained by

Wright's method these cells displayed one or more round to oval nuclei with a moderate amount of chromatin. There was a large amount of uniform blue cytoplasm containing a few unstained vacuoles. Most of these cells were negative to the oxidase reaction although those showing a positive reaction were not uncommon. As the period of incubation increased the difference between these two types of cells became less marked. As observed after a week or two most of the cells seen contained a large amount of phagocyted material and a variable number of refractile granules. So much red-staining material was present in the cytoplasm that the structure of the cells was obscured.

Three general schools exist with different theories as to the source of the large phagocytic cells which develop in incubated blood. Maximow since 1902 has claimed that a primitive cell exists in the tissues and blood which is morphologically like a lymphocyte and which may become an actively phagocytic large cell, a lymphocyte or a granulocyte. Bloom has demonstrated the development of large phagocytic cells in cultures of lymph from the thoracic duct. Since Kindwall and others have shown that lymphocytes are the only cells present in the contents of the thoracic duct it is difficult to conceive of any source for the large cells except cells of the lymphocyte type. Our experiments with cultures of human lymph nodes which will be described in a later paper tend to bear out this theory. Ferrata [20] has described a pluripotential cell which is sometimes present in the blood stream and has called it a hemohistioblast.

The Lewises after extensive observations on bloods of different species of animals concluded that the only source of large phagocytic cells was the blood monocyte. As their large cells phagocyted actively and the engulfed material was irregularly distributed through the cytoplasm they concluded that it was the same as the phagocytic cell of the tissues described by Sabin and called a clasmatocyte. They felt that the monocyte and clasmatocyte were simply functional variations of the same cell.

Sabin and her co-workers explained the development of large phagocytic cells of the clasmatocyte type in incubated blood by stating that endothelial cells or their derivatives were normally present in the blood and gave rise to the large cells. They believed that the monocyte never attained such a large size or phagocyted as actively as the clasmatocyte type cells.

Without discussing the origin of these cells which we will take up in a later paper we have concluded from our observations and from the literature that there are probably two sources for the large phagocytic cell, one, a primitive cell normally present though unrecognizable in the blood; two, the blood monocyte. We think that it is possible for a time to separate the two types of large cells developing from these two sources by certain differences in activity, in staining, in morphology and in phagocytosis. After a certain period of incubation the differences become less marked and many cells of an indeterminate type are seen.

From this premise we feel that the theories of all three schools may be correlated and made to agree with the existing facts which are so difficult to explain on any one theory above.

If the theories of the Lewises were correct, it would be very difficult to account for the oxidase-negative large cells, since ninety-five per cent of human monocytes are oxidase-positive. Should the theories of Sabin, *et al.*, be accepted, the large cells should be oxidase-negative, save for granules of phagocyted material. Neither of these two schools could explain the results of Bloom. Timofejewskij and Benewolenskaja, following the teachings of Maximow, believed the large cells were derived from a primitive, pluripotential cell, the hemocytoblast. Our work would tend to confirm this view. The existence of such a cell would account for the varying oxidase reaction of the large cells and the results of Bloom.

Conclusions

1. The large phagocytic cells developing in incubated rabbit and human blood may be derived from two sources.
2. These large cells show certain differences in morphology, in staining and in activity for a time which make it possible to group them in two classes.
3. One group arises from a primitive pluripotential cell normally present though unrecognizable in the blood stream.
4. The second group develops from the blood monocyte.

REFERENCES

1. Parker, F., Jr., and Rhoads, C. P. *Am. J. Path.*, 1928, iv, 167.
2. Pappenheim, A. *Folia Hemat.*, IV Suppl., 1907, p. 46.
3. Cunningham, R. S., Sabin, F. R., and Doan, C. A. *Contributions to Embryology, No. 16; Publication No. 361 of the Carnegie Institution of Washington*, 1925.
4. Lewis, W. H. The Harvey Lectures of the Harvey Society of New York, 1925–26.
5. Awrorow, P. P., and Timofejewskij, A. D. *Virchows Arch. f. path. Anat.*, 1914, ccxvi, 184.
6. Timofejewskij, A., and Benewolenskaja, S. W. *Virchows Arch. f. path. Anat.*, 1927, cclxiii, 719.
7. Carrel, A., and Ebeling, A. H. *J. Exper. Med.*, 1922, xxxvi, 356.
8. Carrel, A., and Ebeling, A. H. *J. Exper. Med.*, 1926, xliv, 261.
9. Carrel, A., and Ebeling, A. H. *J. Exper. Med.*, 1926, xliv, 285.
10. Maximow, A. *Physiol. Rev.*, 1924, iv, 533.
11. Maximow, A. *Proc. Soc. Exper. Biol. & Med.*, 1927, xxiv, 570.
12. Fischer, A. *Compt. rend. soc. de biol.*, 1925, xcii, 109.
13. Lewis, M. R. *Am. J. Path.*, 1925, i, 91.
14. Lewis, M. R. *Arch. f. Exper. Zellforsch.*, 1926, Bd. 2.
15. Lewis, M. R., and Lewis, W. H. *J. A. M. A.*, 1925, lxxxiv, 798.
16. Lewis, M. R., and Lewis, W. H. *Contributions to Embryology, No. 18; Publication No. 363 of the Carnegie Institution of Washington*, 1926, p. 95.
17. Kindwall, J. A. *Bull. Johns Hopkins Hosp.*, 1927, xl, 39.
18. Bloom, W. *Proc. Soc. Exper. Biol. & Med.*, 1927, xxiv, 567.
19. As given by Sato, A., and Yoshimatsu, S. *Am. J. Dis. Child.*, 1925, xxix, 301.
20. Ferrata, A. *Haematologica*, 1920, i, 243.